A STUDY ON EFFECTS OF SOME PHARMACOLOGICAL AGENTS ON THE PLASMA HALF-LIFE AND HYPOGLYCAEMIC RESPONSE OF TOLBUTAMIDE

THESIS
FOR
DOCTOR OF MEDICINE
(PHARMACOLOGY)

BUNDELKHAND UNIVERSITY
JHANSI (U. P.)

1984

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CERTIFICATE

Certified that the work entitled "A STUDY ON EFFECTS OF SOME PHARMACOLOGICAL AGENTS ON THE PLASMA HALF-LIFE AND HYPOGLYCAEMIC RESPONSE OF TOLBUTAMIDE", has been carried out by Dr. Harendra Kumar himself in this department.

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This is to certify that the work of Dr. Harendra Kumar on "A STUDY ON EFFECTS OF SOME PHARMACOLOGICAL AGENTS ON THE PLASMA HALF-LIFE AND HYPOGLYCAEMIC RESPONSE OF TOLBUTAMIDE", which is being presented by him as a THESIS for M.D.(Pharmacology) has been carried out by the candidate under our guidance and supervision.

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A C K N O W L E D G E M E N T

It is my great pleasure to express my deep sense of gratitude to Prof. D.N. Prasad, M.D., Ph.D., F.I.C.A.I., Principal, M.L.N. Medical College, Jhansi, could spare his precious time from his busy schedules in guiding this research work. His affectionate encouragement helped me very much to complete this work in due time.

I am extremly grateful to Dr. V.K. Kulshrestha, M.D., Professor and Head, Department of Pharmacology, for his valuable suggestions. He helped me to solve many problems which arose during this work.

I express my heartfelt gratitude to Dr. M.B. Misra, M.D., Reader in Pharmacology for His encouraging suggestions about this research work from time to time.

Helpful suggestions of Dr. V.V. Sharma, M.D., Lecturer, Department of Pharmacology, is very much appreciable. I will remain indebted to him for his immense help in completing this work.

I express my gratitude to Dr. S.B. Nayak, M.D., Lecturer, Department of Pharmacology, and his family, who always gave me time to make the work fruitful. Dr. Nayak who incited me to pursue this research work is to be acknowledged here with gratitude. He discussed each and

every thing with great care and interest.

Thanks are due to Dr.(Mrs.) Sadhna Kaushik, Dr. Rajesh Gupta, Dr. Pradeep Kumar Dubey, Dr. Anil Nigam, Dr. R. N. Das, Dr. Harish Mittal, Dr. Sunil Gupta, Mr. I.B. Singh, Mr. Nand Nathani, Mr. Rajeev Gupta, Mr. Arvind Kumar Vaish and Mr. V.N. Misra for their help at various steps in this study.

The help rendered by Mr. O.P. Shukla, Mr.S.K. Gupta, Mr. G.S. Shukla, Mr. K.V. Srivastava, Mr. Dhanna Lal are worth remembering. Thanks are due to Mr. Laxman Mr. Bhagwan Das, Mr. Jai Narain, Mr. Hari Ram and Mr. Anwar for their assistance.

Typing work of Mr. P. C. Sadhan, Mr. K.M. Thomas and Miss Shobha P. is to be acknowledged.

Accomplishment of this work could only be possible by affectionate love and constant inspiration from my parents and other family members.

I do not find way, how to apologise for the pain given to innocent animals during this research work for the sake of mankind.

30th May 1983

JHANSI

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INTRODUCTION

I N T R O D U C T I O N

Multiple medication has become common feature in most prescriptions in modern medical practice. Concommitant use of one drug may alter the intensity of pharmacological effect(s) of another drugs. Concurrent use of multiple drugs some times produces beneficial interactions and is often essential to obtain a desired therapeutic objective. But on most occasions such medication produces harmful side effects. The frequency of significant beneficial or adverse drug interactions is unknown. Survey that include data obtained in vitro, in animals and in case reports tends to predict a frequency of interaction that is higher than that actually occurs. While such reports have contributed to skepticism about the overall importance of drug interactions (Koch - Weser and Green Blatt, 1977), the physician must be alert for their occurrence.

When a diabetic individual remains untreated or not adequately treated cardiovascular, neurological renal and retinal complications arise in the future clinical course (Foster, 1980).

Due to reduced body resistance diabetic patients are always prone to various microbial infections (Foster, 1980).

In the medical management of diabetes mellitus a physician always faces multiprong problems particularly in the treatment of associated complications. Prescription of multiple medications along with insulin and/or oral antidiabetic agents is a clinical problem to physicians due to drug interacting potentialities.

Beta-adrenoceptor blockers and nonsteroidal anti-inflammatory analgesics are very commonly prescribed for the treatment of associated hypertension, occlusive coronary diseases and pain arising from diabetic ulcers and other inflammatory diseases. A thorough knowledge of drug interactions particularly of various common groups of drugs with antidiabetic agents is necessary to prevent any possible side effects arising from use of their concomitant administration. Anti-inflammatory agents and beta-blocking drugs are known to produce drug interactions with sulphonylureas (Hansten, 1979). Inspite of large number of reports the mechanism of interactions still remains unexplored. In course of time due to discovery of newer drugs and replacement of older drugs the clinicians have to be alert for their interaction. At present many

agents have been recently introduced in clinical therapy. Studies on these drugs with antidiabetics are very much limited.

In the present study tolbutamide was selected among the sulphonylureas because of its low toxicity higher safety and high clinical efficacy besides it can be estimated by standard procedure in the blood. For interaction studies with tolbutamide, aspirin, tolmetin and tromaril among the anti-inflammatory drugs and propranolol, atenolol, metoprolol and acebutolol among the beta-adrenoceptor blockers have been selected for this study.

For interaction study with tolbutamide blood sugar estimation has been used as the major parameter but to make the study more conclusive the serum tolbutamide measurements have been also made.

The present study was undertaken with the following aims in view.

(1) To confirm the hypoglycaemic effect of tolbutamide in normal and experimentally induced (alloxan) diabetic rabbits and to select a suitable dose of tolbutamide for further interaction studies.

(2) To study the effect of anti-inflammatory agents after single and repeated treatment on tolbutamide

induced hypoglycaemia, corresponding serum tolbutamide concentrations and tolbutamide biological half-life in normal and diabetic rabbits.

(3) To study the effect of beta-blocking agents after single or repeated treatment in normal and diabetic animals on tolbutamide-hypoglycaemia and corresponding serum tolbutamide concentration and its half-life.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Since single drug prescriptions have become rare in current medical practice, the chances of drug-drug interactions at present have increased considerably. That many of these drug combinations have the potential to interact adversely (Hansten, 1979). Gravity of adverse effects due to drug interaction is not fully known because of limited work done to explore interacting possibilities. Most of the work done to know the drug-drug interactions is limited to easily measurable parameters. Mechanism of many already reported drug-drug interactions are not well understood. However, changes in metabolism of interacting drugs may tell something about the mechanisms of interactions.

Fortunately the subject of drug interactions has developed a new field of interest in pharmacological research. Knowledge of drug interactions enables a physician to minimise or prevent drug toxicity by adjustment of dosage or schedule of drug administration or by choice of an alternative agent.

Drug interactions may occur by multiple mechanisms. Though every mechanism is of its own kind, even then, leaving a few exceptions they can be classified

as follows according to Cohen and Armstrong (1974).

(1) Interactions dependent on gastro-intestinal absorption.

(2) Interaction between drugs at their plasma protein binding sites.

(3) Interaction due to altered drug metabolism which may be

(a) increased
or
(b) decreased

(4) Interaction resulting from altered renal excretion of a drug or its metabolites.

(a) Increased Excretion

(b) Decreased Excretion

(5) Interaction at drug receptor site.

(6) Direct physical or chemical interaction between concurrently administered drugs.

(7) Undefined mechanisms.

Pharmaceutical interference may occur between drugs that are included in the same intravenous(I.V.) solution. Such interference is strongly dependent upon drug concentration and on the ionic properties and/or pH of the IV solution and is often influenced by "filler" or stabilizing substances that may be added to pharmaceutical preparations.

ABSORPTION INTERACTIONS

The rate of absorption of orally administered drugs is largely determined by the rate of gastric emptying (Prescott, 1974), the nature of gastric contents (volume, composition and pH), pathological states and physico-chemical properties of drugs. Likewise different mechanisms have been suggested to explain the drug interaction at the level of absorption which can be summarised as follows :

(1) Effect of pH of gastrointestinal fluid on drug dissolution rate and/or solubility.

(2) Pharmacological interference by drugs with active transport mechanisms involved in the absorption of other drugs.

(3) Formation of drug-drug complex or ion-drug complexes which may either enhance or retard drug absorption.

(4) Interference with gastrointestinal enzymes involved in drug absorption.

(5) Effects of certain drugs on gastric emptying rate and/or gastrointestinal motility.

(6) Direct toxic effects of drugs on gastro-intestinal flora.

pH EFFECTS

In order to be absorbed, drugs must pass

through the lipoprotein membrane of cells that line the gastrointestinal lumen. The rate of diffusion across the membrane is affected by the state of ionization of the drug. Nonionized drugs are usually more lipid soluble and thus diffuse across the cell membranes more readily. At the normal acid pH of the stomach, basic drugs such as amphetamine, quinidine, chloroquine are highly ionized and thus are poorly absorbed. Drugs that are weak acids, such as aspirin, phenylbutazone and phenobarbital are less highly ionized at the pH of normal gastric fluid and are, therefore, more lipid soluble. Antacids by raising the intraluminal pH of the stomach, increase the ionization of acidic drugs. Conversely by raising the intraluminal pH of stomach, antacids decrease ionization of basic drugs and thereby increase their absorption (Cohen and armstrong, 1974).

Elevation of stomach pH by antacids has also been shown to delay gastric emptying of food and drugs, and thus may either increase or decrease absorption. depending upon the site of absorption of the drug primarily from the stomach or from the intestine. In addition, the pH of the stomach and other organs of the gastrointestinal tract can affect absorption of

drugs by altering the solubility or stability of the drug. For example, oral penicillin G is degraded rapidly at the normally acid pH of the stomach, but degradation is decreased and absorption is consequently increased when an antacid is administered concurrently (Cohen and Armstrong, 1974).

The rate of absorption of salicylates, indomethacin, naproxen, pseudoephedrine, sulphadiazine and enteric-coated phenylbutazone or aspirin is increased at elevated pH. The absorption of dicumarol/but not of warfarin, is also facilitated by the formation of a rapidly absorbable complex. Aluminium hydroxide accelerates the absorption and increases the bioavailability of diazepam by an unknown mechanism (Gilman et al., 1980)

Other factors influencing drug absorption

Since most drugs are absorbed more slowly from the stomach than from the small intestine, the rate of gastric emptying can be an important factor in influencing drug absorption. Cathartics may reduce uptake of poorly absorbed medications from the small intestine as a consequence of their effects on gastrointestinal motility. Surface acting agents such as charcoal can bind various drugs in the gastrointestinal tract and decrease their absorption. Agents which reduce lipid absorption

(e.g. cholestyramine) may also interfere with the absorption of lipid soluble drugs. The absorption of certain pharmacologically active agents (e.g. folic acid) is accomplished by enzymedependent transport mechanisms operating in the gastrointestinal mucosa and these mechanisms can be affected by concurrent administration of various drugs (Cohen and Armstrong, 1974).

Drugs which alter the intestinal flora may necessiate change in dose and dose intervals of certain drugs. e.g. that after sterilisation of gut following neomycin,oral anticoagulants have exaggerated effect and methotrexate produces toxicity (Zaharka et al., 1969).

Salts of aluminium, calcium, magnesium and iron all chelate with tetracyclines and impair their absorption (Neuvonen et al., 1961; Kunin and Finland, 1970). These interactions, however, occur only if the interacting agents are administered simultaneously or within 30 to 60 minutes of each other.

Bioavailability of a number of drugs is decreased because of their capacity to form complexes with various antacids. Magnesium trisilicate and silicon dioxide formed there from strongly bind and interfere

with bioavailability of iron, digoxin, certain benzodiazepines and phenothiazine. Aluminium hydroxide decreases the bioavailability of propranolol, antimuscarinic drugs, digoxin, chlorpromazine and sulphadiazine (Gilman et al., 1980)

DRUG DISPLACEMENT FROM PLASMA PROTEIN BINDING SITES:

A fair number of drugs, especially those that are acidic, are reversibly bound to plasma or tissue proteins and the extent of competition between drugs for such binding sites depends on the affinity of each for the site and its concentration. These drug binding proteins function as storage site for the drug; the pharmacologically active unbound fraction of the drug is in equilibrium with the bound fraction which is pharmacologically inert. It is the unbound fraction that has access to the cellular receptor sites where the drug exerts its pharmacological effects. In addition, the unbound fraction is subject to clearance from the body by metabolism and/ or excretion.

In instances where drugs are very highly bound to plasma protein (e.g. 90 to 98 % bound), only a small fraction of the total circulating drug(i.e. the 2-10 % of the drug that remains unbound) is responsible for

pharmacological activity. In such a case, even a small decrease in plasma protein binding can lead to a doubling or tripling of the unbound fraction of the drug. The resulting increase in pharmacological activity is usually temporary, as rapid clearance from the circulation takes place and a new equilibrium is formed. Nevertheless even a temporary elevation of levels of pharmacologically active drugs may sometimes lead to demonstrable clinical consequences

ALTERATION IN DRUG METABOLISM FROM ADMINISTRATION OF OTHER DRUGS:

Increased Metabolism

Most of the drugs are metabolised in hepatic microsomes with the help of different enzymes. It is now well recognised that various chemicals can increase (induce) the synthesis of microsomal drug metabolising enzymes in various animal species. In many instances as increased rate of drug metabolism leades to decreased pharmacologic action, however, in some instances where the metabolite of a drug is more active than the parent compound, enzyme induction can lead to an increase in pharmacologic activity of the drug(Cohen and Armstrong, (1974).

Chlordiazipoxide, chlorpromazine, hexobarbital, meprobamate, phenobarbital, phenylbutazone, probenecid, and tolbutamide are some examples of drugs which enhance their own metabolism(Melmon and Morrelli, 1972).

And there are some agents which enhance metabolism of other substances (Table - 1) by inducing hepatic microsomal enzymes.

Table No. - 1 : Drugs that enhance the metabolism of other drugs or substances.

Inducing agent	Drugs or substances affected
Phenobarbital	Barbiturates Phenylbutazone Warfarin Griseofulvin
Diphenylhydantoin	Corticosteroids and Steroid hormones.
Chlorcyclizine	Corticosteroids and sex hormones
Norchlorcyclizine	Corticosteroids and sex hormones
Orphenadrine	Corticosteroids and sex hormones
Phenylbutazone	Corticosteroids and sex hormones
Amobarbital	Warfarin
Alcohol	Tolbutamide

Decreased Metabolism

Certain drugs inhibit the activity of

enzymes responsible for the metabolism of other drugs. Such inhibition may result from competition between the pharmacologic agents able to act as substrates for the same drug metabolizing enzyme, or from direct interference with the enzyme itself.

Table No. - 2 : Following are the drugs which are thought to interact apparently by inhibiting other drugs metabolism(Melmon and morrelli, 1972; Girdwood, 1976).

Drugs metabolised slowly	Drugs inhibiting metabolism
Bishydroxycoumarin	Chloramphenicol, oxyphenbutazone and phenylbutazone.
Diphenylhydantoin	alcohol, p-aminosalicylic acid, bishydroxycourarin, chloramphenicol, cycloserine, diazepam, INH, PAS, phenylbutazone, phenobarbitone, phenyramidol, probenecid, sulphaphenazole and sulthiame.
Tobutamide	alcohol, chloramphenicol, dicoumarol, MAO-inhibitors, phenylbutazone, phenyramidol, probenecid, salicylates and sulphaphenazole.
Nortriptyline	hydrocorisone, perphenazine.

INTERACTION AT LEVEL OF EXCRETION

Drugs which have the ability to increase or decrease glomerular filtration by altering renal blood

flow may alter the rate of excretion of other drugs or metabolites theoritically. However, there is little clinical evidence of interaction by this mechanism.

Sulfinpyrazone in sufficient dosage is a potent inhibitor of the renal tubular reabsorption of uric acid. As with other uricosuric agents, small doses may reduce the excretion of uric acid, like probenecid, sulfinpyrazone reduces the renal tubular secretion of many other organic ions. The drug may induce hypoglycaemia by decreasing the excretion of the sulphonylureas (Mudge, 1980). The uricosuric action of sulfinpyrazone is additive to that of probenecid and phenylbutazone but is mutually antagonistic to that of the salicylates (Yii et al., 1963).

Table No. - 3 : Important interactions at the level of excretion are given as follows(Girdwood, 1976).

Drugs	Delay(s) excretion of
Probenecid	Dapsone, Indomethacin, PAS, Sulphinpyrazone, penicillins, Cephalothin, Cephalexin.
Dicoumarol, phenylbutazone	Chlorpropamide
Salicylates, sulphonamides.	Methotrexate

INTERACTION AT DRUG RECEPTOR SITE

This mechanism of drug interaction involves competition for receptors at the cellular site where the

drugs ultimately exert their pharmacologic effects. Unlike plasma protein binding sites cellular receptor sites for drugs are usually highly specific. Competition may result from the blocking of a receptor site by another drug. In addition, competition for specific uptake mechanisms may also occur. A well studied example of this involves blockade of the norepinephrine pump by tricyclic antidepressants. Since uptake of guanethidine by the NE pump at the adrenergic nerve ending is required in order to exert its antihypertensive effect, competition for the uptake mechanism by tricyclic antidepressants renders guanethidine ineffective as an antihypertensive agent (Cohen and Armstrong, 1974).

Other interactions of an apparent pharmacodynamic nature are poorly understood. Halogenated hydrocarbons, including many general anaesthetics, sensitise the myocardium to the arrhythmogenic actions of catecholamines. This effect presumably results from some action on the pathway leading from receptor to effector, but details are not clear. Many signs and symptoms of hypoglycaemia are mediated through the adrenergic nervous system and are masked by beta-adrenergic blocking agents. Patients taking propranolol may thus fail to note reactions to insulin or oral hypoglycaemic agents in time to prevent

dangerous consequences and further more, compensatory mechanisms, such as glycogenolysis, may be blocked by the beta-adrenergic antagonists (Melmon and Gilman, 1980).

ORAL ANTIDIABETIC AGENTS

The search for natural remedies for diabetes has been persistent as in most chronic ailments. Between 1918 and 1930 many compounds were tested as oral anti-diabetics e.g. guanidine (Watanabe, 1918), synthalin A and Synthalin B (Frank et al., 1928) but failed to survive as therapeutic agent due to their high toxicity.

Janbon and coworkers (1942) in the course of clinical studies on the treatment of typhoid fever, discovered that a sulphonamide (p-aminobenzene - Sulpho-namide - isopropylthiadiazole) induced hypoglycaemia. Loubatiers (1957) then made a fundamental discovery that the compound exerted no hypoglycaemic effects and suggested that the action was the result of stimulation of pancreas to secrete insulin. Franke and Fuchs (1956) reported the use of carbutamide (BZ55), a sulpholyurea compound and found that it could be successfully substituted for insulin in a number of middle aged elderly diabetic. Soon thereafter, the compound tolbutamide was introduced. The tolbutamide proved to be less toxic than carbutamide

and soon became popular for the management of certain diabetic patients

Another group of compounds, the biguanides was developed independently of sulphonylureas. Historically the development began with the discovery in 1918 by Watanabe that guanidine causes hypoglycaemia in rats. Subsequently the compound phenformin was introduced into clinical therapy and was used for several years. Now it has been replaced by better drugs.

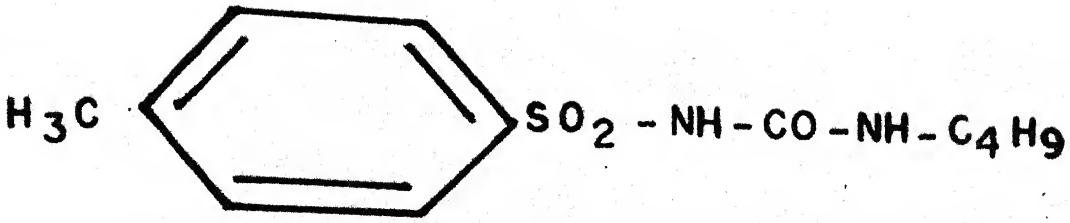
ORAL HYPOGLYCAEMIC AGENTS IN CURRENT USE

Sulphonylureas and biguanides are the two classes of drugs used as oral hypoglycaemic agents. Mechanism of action of biguanides is entirely different from those of sulphonylureas. A large number of sulphonylureas derivatives have been studied. All are synthetic and have the same basic mechanism of action. They differ in metabolic fate, potency and toxicity. The most important difference among the sulphonylureas for clinical purpose, is in their duration of action. In increasing order they are tolbutamide, tolazamide, glibenclamide, acetohexamide and chlorpropamide (Meyers et al., 1976)

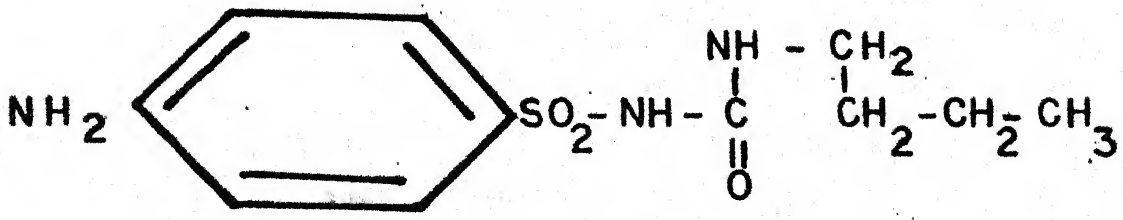
PHARMACOLOGY OF TOLBUTAMIDE:

Chemistry:

All sulphonylurea drugs are arylsulphonylureas with substitutions on the benzene and the urea groups.



TOLBUTAMIDE



CARBUTAMIDE

Fig. 1 : Shows chemical structure of tolbutamide, N-(P-tolyl sulphonyl) - N-butyl carbamide; and carbutamide, N-Sulphanilyl-N-butylcarbamide.

In the case of tolbutamide (Fig. 1) aryl group is tuolyl and the urea substitution is butyl. Tolbutamide differs from antibacterial compound carbutamide in having methyl instead of amino on the benzene ring. This substitution accounts for the loss of antibacterial properties and for the reduction of toxicity (Larner, 1980).

Physical Properties:

It is a white odourless powder with acid pH, soluble in alcohol and insoluble in water. It is soluble in alkaline intestinal contents of human beings and carnivorous animals (Shaw and Bosser, 1971). Tolbutamide is readily soluble in amylacetate which is used for estimation of tolbutamide in biological fluids(Spingler, 1957).

MECHANISM OF ACTION:

Tolbutamide stimulates the islet tissue to secrete insulin like other sulphonylureas. Administration of sulphonylureas increases the concentration of insulin in the pancreatic vein in cross circulation experiments (Larner, 1980). The stimulating effect of tolbutamide on insulin release can be demonstrated in vitro and invivo-experiments in normal animals and human beings. This is demonstrated histologically by peripheral migration and

discharge of beta - granules (Williamson et al., 1961). Furthermore, this stimulating effect is dependent on the functional state of beta-cell reserve (Pfeiffer, 1967). The action of the drug requires a minimum amount (atleast 30 % of normal) of functioning beta-cell tissue. This effect does not occur in pancreatectomized individuals or patients with an absolute difficiency of insulin like Juvenile diabetes (Shar and Beaser, 1971). Hallman and associates (1971) concluded that labeled tolbutamide is restricted in its action to the extracellular spac and does not need to enter the beta cells. The invoked release of insulin is immediate and is intimately related to the action of glucose. The drugs may sensitize the cell to the normal secretagogue.

In experimental animals and in diabetic patients conflicting results have been obtained on the effects of tolbutamide on the plasma concentration of glucagon. Samols and Harrison (1976) have suggested that tolbutamide can enhance glucagon secretion from the alfa-cells, although this may be masked by the effect of sulphonylureas to stimulate the secretion of insulin. Local actions of insulin within islet may cause a reduction in the secretion of glucagon; the net effect may be either stimulation or suppression of glucagon secretion.

During chronic administration, a significant portion of hypoglycaemic action of the sulphonylureas may be due to extrapancreatic actions. Insulin biosynthesis may be actually decreased and peripheral tissues become more sensitive to a fixed dose of administered hormone due possibly to an increase in the number of insulin receptors(Lebovitz and Feinglos, 1978). Tolbutamide enhances the antilipolytic action of insulin in adipose tissue. This appears to be related to an altered effectiveness of cyclic AMP rather than to any change in metabolism of cyclic nucleotide (Brown et al., 1972; Fain et al., 1972) and an inhibitory effect of the drug on cyclic AMP- dependent protein kinase has been observed (Wray and Harris, 1973). A reduction in the hepatic uptake of endogenous insulin has been described (Marshall et al., 1970) and a direct inhibitory effect of tolbutamide on hepatic glucose production may also be demonstrated in the presence of insulin (Shambye and Tarding, 1989).

PHARMACOKINETICS OF TOLBUTAMIDE

ABSORPTION

When administered orally, tolbutamide is absorved promptly from the small intestine (Danowski, 1966) and appears in blood within 30 minutes. Its peak concentration is attained in 3 to 5 hours(Larner, 1980)

The availability is 93 % ± 10 % when given orally (Nelson and O'Reilly, 1961).

DISTRIBUTION

Tolbutamide is distributed throughout the extracellular fluid compartment so the volume of distribution of tolbutamide is approximately equal to the extracellular fluid (Mahe et al., 1962). Williams et al. (1977) calculated the volume of distribution of tolbutamide to be 0.15 ± 0.03 litres/kg. 93 % ± 1 % of tolbutamide is bound to plasma proteins which may decrease in acute viral hepatitis (Williams et al., 1977).

Table 4

Pharmacokinetic data of tolbutamide(Gilman et al.,1980)

Availability (oral) (%)	Urinary excretion (%)	Bound in plasma %	Clearance ml min^{-1} kg^{-1}
93 ± 10	NIL	93 ± 1 decrease in AVH	0.30 ± 0.5 increased in AVH

Vol. Dist. (litres/kg)	Half-life (hours)	Effective concentration	Toxic concentration
0.15 ± 0.03 No change in AVH	5.9 ± 1.4 decreased in AVH, CRI, No change in aged and Uraemia	80-240 µg/ml	-

AVH = Acute Viral Hepatitis, Vol.Dist = Volume of distribution
CRI = Chronic respiratory insufficiency.

BIOTRANSFORMATION

Tolbutamide is oxidised in liver. It is first converted into hydroxytolbutamide which is partially excreted unchanged and the majority is further oxidised to carboxytolbutamide which is finally excreted. The oxidation of tolbutamide is the rate limiting step in the elimination of the drug and its metabolites. Subsequent oxidation steps are very rapid. Accordingly a short time after tolbutamide administration, the rate of excretion of the sum of the two metabolites equals the rate of tolbutamide oxidation and offer a very sensitive measure of changing tolbutamide oxidation(Rowland, 1974).

HALF-LIFE

The biological half-life of tolbutamide (defined as the time required for the blood level to decrease from the peak level by 50 %) is 6.6 hours. The metabolic half-life (defined as half the interval of blood sugar lowering effect) is 4.7 hours(Shaw and Beaser, 1971).

Williams et al.(1977) reported that half-life of tolbutamide in normal individuals is 5.9 ± 1.4 hours which was significantly decreased in acute viral hepatitis, to 4.00 ± 0.9 hours.

TOXICITY OF TOLBUTAMIDE

The enormous use of sulphonylureas has confirmed their conspicuous freedom from serious side effects, Bloom (1959) has described tolbutamide to be the safest drug to be introduced after a long time.

Toxicity tests in animals have shown that in ordinary doses tolbutamide has no action on respiration, circulation or on the smooth muscles of the gut and does not affect the contraction of uterus produced by histamine and ergotamine(Oakley, 1958).

O'Donovan (1959) analysed the incidence of side effects of tolbutamide in 9188 cases. The total incidence of side effects was 3.2 %; the drug had to be withdrawn in 1.6 % of the patients. The reactions have been classified as haematological (0.24 %), cutaneous (1.1 %) and gastro-intestinal (1.4 %) of the 22 subjects exhibiting haematological abnormalities, 19 had transient leucopenia; in 9 instances, the leucocyte count returned to normal despite continuation of the drug.

HYPOGLYCAEMIA

Hypoglycaemia, although relatively uncommon is still a significant complication. Severe fatal hypoglycaemic attack may occur which is refractory to treatment (Cushman et al., 1963).

GASTROINTESTINAL DISTURBANCES

In susceptible individuals, symptoms consist of heartburn, upper abdominal discomfort, nausea, lower abdominal cramps and diarrhoea. According to Malins (1968) gastrointestinal upset occurs in more than 6 % cases treated with tolbutamide.

SKIN RASHES:

The rash has the usual feature of drug eruption and clears rapidly when sulphonylureas is withdrawn. Skin reshes may be seen in 3 % cases taking tolbutamide (Malins, 1968).

LIVER FUNCTION

Rarely cholestatic jaundice may occur after the use of tolbutamide (Baird and Hull, 1960). On very rare occasions tolbutamide may aggravate hepatic porphyria (Schalsinger and Gastel, 1961).

PANCYTOPENIA

Pancytopenia was reported following tolbutamide administration (Chapman and Cheung, 1963).

ALCOHOL INTOLERANCE

This consists of intense flushing of the face and malaise immediately after taking even very small amount

of alcohol with sulphonylureas. The incidence of the reaction is less with tolbutamide than chlorpropamide (Malins, 1968).

Antithyroid action

Brown and Soloman (1956) showed a fall in the I^{131} uptake and protein bound iodine levels in diabetics taking carbutamide and tolbutamide.

GLUCOSE METABOLISM

Final products of carbohydrate digestion in the alimentary tract are almost entirely glucose, fructose and galactose with glucose representing on the average about 80 % of these monosaccharides. After absorption from the intestinal tract, most of the fructose and galactose are almost immediately converted into glucose. Therefore, very little fructose and galactose are present in the circulating blood. Glucose thus becomes the final common pathway for transport of almost all carbohydrates to the tissue cells. In liver cells, appropriate enzymes are available to promote interconversion among the monosaccharides before glucose can be used by the cells. Glucose is transported through the cell membrance by the mechanism of facilitated diffusion. The rate of glucose transport and also transport of some other monosaccharides is greatly increased by insulin with

the exception of the liver and the brain(Guyton, 1981). Immediately upon entry into the cells glucose combines with a phosphate radical to form glucose 6-phosphate. The phosphorylation promoted by glucokinase is almost completely irreversible except in the liver cells, the renal tubular epithelium and the intestinal epithelial cells in which glucose phophatase is available for reversing the reaction. Therefore, in most tissues of the body phosphorylation serves to capture glucose in the cell.

GLUCOSE - INDUCED INSULIN SECRETION

Glucose stimulates insulin secretion in man, monkey (Kriss et al., 1966), rabbit (Coore and Randle, 1964) and rat (Grodsky et al., 1963). The rapidity of the insulin secretory response to glucose is best illustrated in vivo or in the perfused isolated pancreas(Curry et al., 1968; Grodsky et al., 1967; Kanazawa et al., 1968), but is also observed in a non-irrigated tissue. The secretory process undoubtedly consumes energy (Malaisse et al., 1967; Ronals, 1970).

CATIONS AND INSULIN SECRETION

Basal or glucose induced insulin release is enhanced whenever sodium influx into the beta-cells is increased (Hales and Milner, 1968; Milaisse et al., 1971;

Milner and Hales, 1967), Diphenyl hydantoin abolished glucose - induced secretion in vivo (Peters and Semaan, 1969) or in vitro (Levin et al., 1970), apparently by inhibiting Na^+ entry into the beta-cell (Kizer and Bressler, 1969). Moreover glucose-induced secretion is inhibited by replacement of Sodium ion by lithium ion (Milner and Hales, 1967) and stimulation of insulin secretion is accompanied by beta-cell depolarization (Dean and Mathews, 1968). These convergent observations support the concept that Na^+ influx into the beta-cell is a significant event in the process of insulin release (Hales and Milner, 1968).

Calcium requirements for insulin secretion

The presence of extracellular Calcium is required for glucose or any other insulinotropic agents to stimulate insulin secretion (Curry et al., 1968; Grodsky and Bennet, 1966). Barium ion can be substituted for calcium ion (Malaisse et al., 1970; Milner and Hales, 1968). By contrast magnesium ion in high concentration inhibits glucose-induced insulin release (Bennet et al., 1969).

In view of the analogy between stimulus secretion coupling in the beta-cell and excitation-contraction coupling in the muscles, it is tempting to speculate that

Calcium ion induces insulin release by causing the contraction of the microtubular-microfilamentous system (Malaisse, 1972).

THE ADRENERGIC MECHANISMS:

In 1964 Coore and Randle observed inhibition of glucose-induced insulin secretion by epinepherinein incubated pieces of rabbit pancreas. Inhibitory effect of epinepherine on insulin secretion has also been confirmed in man(Karam et al.,1966) and rat (Malaisse et al., 1967).

The inhibitory effect of epinepherine is not restricted to the insulinotropic effect of glucose. Thus epinepherine also abolishes secretion in response to glucagon (Porte et al., 1966), theophylline(Malaisse et al., 1970), tolbutamide (Malaisse, 1967; Porte et al., 1966), aminoacids (Hartelendy, et al., 1968).

Epinepherine is a more potent inhibitor of insulin secretion than norepinepherine (Malaisse et al., 1967; Porte and Williams, 1966). Because epinepherine is also the most potent activator of alpha adrenergic receptors, these findings suggest that epinepherine-induced inhibition of insulin secretion results from the activation of alph-adrenergic receptors. The hypothesis is substantiated by the fact that alph-adrenergic blocking agents abolish the inhibitory effect of adrenaline, whereas, beta-adrenergic

blocking agents fail to do so (Porte, 1967).

Porte (1967) first reported elevation in the level of circulating insulin during infusion of isoproterenol in human subjects. Orciprenaline has the same effect (Laudicina et al., 1968). In vitro, although beta-adrenergic blocking agents might also exert some inhibitory effect under appropriate experimental conditions(Malaisse et al., 1967), they do not suppress glucose-induced insulin secretion (Malaisse et al., 1967). Effects of different beta-blockers on glucose metabolism have been discussed subsequently.

CHOLINERGIC MECHANISMS

The direct stimulant effect of parasympathomimetic drugs on the beta-cell was confirmed in vivo in dog and man (Kajinuma et al., 1968; Kaneto et al., 1968). In these species the enhanced insulin output evoked by cholinergic agents could be antagonised by atropine (Prohman et al., 1967).

EFFECTS OF ANTIINFLAMMATORY AGENTS ON GLUCOSE METABOLISM:

Salicylates:

The effects of salicylates on carbohydrate metabolism are complex. Multiple factors appear to be involved, some tending to lower and others to raise the

blood glucose concentration. In both animals and man, large doses of salicylates may cause hyperglycaemia and glycosuria and deplete muscle and liver glycogen. These effects are partly explained by the release of epinephrine through activation of central sympathetic centers. In addition, such large doses might reduce aerobic metabolism of glucose, increase glucose-6-phosphatase activity and promote the secretion of glucocorticoides (Flower et al., 1980; Pickering, 1968). Hypoglycaemic action of salicylates may be seen in diabetic or nondiabetic patients having taken toxic doses of salicylates (Hanston, 1979).

PHENYLBUTAZONE:

Phenylbutazone although does not produce any marked change in blood sugar independently(Sharma et al., 1981) but potentiates hypoglycaemic effect of insulin (Flower et al., 1980).

INDOMETHACIN

Indomethacin in rare occasions produces hyperglycaemia and glycosuria. However, in most studies indomethacin did not affect glucose tolerance (Rothermich, 1966).

TOLMETIN

It is a comparatively new anti-inflammatory agent. This drug has been seen to produce significant hypoglycaemia

in rats and rabbits (Sharma et al., 1982). The mechanism by which it produces this effect has not been elucidated.

TROMARIL

This is latest drug in the series of anti-inflamatory agents. It is an anthranillic acid derivative claimed to be safer anti-inflammatory drug(Mathur et al., 1980; Sattur et al., 1980). Although, a large number of studies indicate that the drug has least toxic effects with high margin of safety, its biochemical effects are still not well studied.

IBUROPEN

Effects of iburopen on glucose metabolism are not well studied. In one study iburopen (10 mg/kg) produced hyperglycaemia in rabbits(Sharma et al.,1981).

MODE OF ACTION OF ANTI-INFLAMMATORY DRUGS ON GLUCOSE METABOLISM

The literature on this aspect does not depict a clear picture. Some of the anti-inflammatory agents (salicylates in toxic doses, indomethacin, ibuprofen) evoke a hyperglycaemic response (Rothermich, 1966; Flower et al., 1980; Sharma et al., 1981) whereas tolmetin and phenylbutazone produce hypoglycaemia (Flower et al., 1980 Sharma et al., 1982). Anti-inflammatory agents are beyond doubt potent prostaglandin synthesis inhibitors, thereby,

produce various pharmacological actions (Smith and Willis, 1971). PGEs also have some insulin like effects on carbohydrate metabolism (Nakano, 1973) and stimulates insulin release (Johnson et al., 1973). PGEs, if really play such role, its inhibition is likely to be accompanied by hyperglycaemic response. But this effect could be modified by centrogenic involvements (Flower et al., 1980) leading to hypoglycaemic or hyperglycaemic effects.

EFFECTS OF BETA-ADRENERGIC BLOCKERS ON GLUCOSE METABOLISM

Propranolol acts synergistically with insulin in the rat to induce hypoglycaemia much more severely (Myers and Friedman, 1966). Cases have been reported where hypoglycaemia has been associated with the use of nonselective beta-adrenoceptor antagonists(Propranolol) in insulin dependent diabetics (Kotler et al., 1966; Revano and Rosenbaum, 1968) and evidence exists that there is a delayed recovery from insulin - induced hypoglycaemia with propranolol (Deacon et al., 1977).

Further investigations suggests that the cardioselective agents atenolol(Deacon et al., 1977) and metoprolol (Newman, 1976) have little or no effect on recovery from insulin - induced hypoglycaemia. A recent study has shown that in insulin - dependent diabetic patients neither

metoprolol nor oxprenolol affected the recovery from hypoglycaemia (Keen et al., 1979).

The metabolic response to hypoglycaemia involves the mobilization of FFA(Free fatty acids) and lactate, both of which are reduced by propranolol, so that a hypoglycaemic tendency is enhanced in the presence of propranolol(Fitzgerald, 1980). In contrast to previous study, Weaver (1980) has reported that metoprolol may impair glucose tolerance in diabetic patients and perhaps in normal individuals. It seems clear that nonselective beta-blocking agents are more likely to affect glucose metabolism and induce hypoglycaemia. The newer cardioselective betablockers affect blood sugar level less adversely. However, in patients with hypertension and mild diabetes a change of therapy from nonselective beta-adrenoceptor antagonists to metoprolol resulted in a significant improvement of glucose tolerance in 6 of 17 patients (Weal-Manning, 1976). The effect of acebutolol(a cardioselective beta-adrenoceptor blocker) on plasma glucose level has also been studied in both normal volunteers and in diabetics. In general little action has been observed on the glucose level (Gibbons et al., 1976; Deacon, 1977; Deacon et al., 1977) or on insulin secretion(Hamm et al., 1978), but Neuman (1976) has noted a potentiation of the effects of insulin and a delay in recovery of the normal glucose level after administration of acebutolol.

INTERACTIONS OF ANTI-INFLAMMATORY AGENTS WITH TOLBUTAMIDE

Phenylbutazone:-

Phenylbutazone enhances the effect of sulphonylureas and the possible mechanism responsible for the effect is the combination of increased sulphonylurea - induced insulin release (Flower et al., 1980), inhibition of metabolism (Pond et al., 1977; Christenson et al., 1963) of tolbutamide and inhibition of excretion of active metabolites (Field et al., 1967). Displacement of tolbutamide from plasma protein binding by phenylbutazone may also be involved in the enhanced hypoglycaemic effect of tolbutamide.

Salicylates:

Salicylate may potentiate the sulphonylurea induced hypoglycaemia due to their intrinsic hypoglycaemic action (Flower et al., 1980). In vitro studies have shown sodium salicylate to displace tolbutamide and chlorpropamide from plasma protein binding thus increasing unbound (active) sulphonylurea. It has also been proposed that salicylates might interfere with the renal tubular secretion of chlorpropamide (Hansten, 1979).

Tolmetin

It has been shown that tolmetin potentiates glibenclamide-induced hypoglycaemia in rabbits (Sharma et al, 1982).

Ibuprofen

Ibuprofen antagonises glibenclamide - induced hypoglycaemia in rabbits(Sharma et al., 1981).

INTERACTIONS OF BETA-ADRENERGIC BLOCKERS WITH TOLBUTAMIDE

It is shown that propranolol blunts the rebound of serum glucose following insulin-induced hypoglycaemia. The effect of propranolol on sulphonylurea-hypoglycaemia is less clear. In one study conducted on healthy subjects, propranolol impaired tolbutamide induced hypoglycaemic response presumably due to inhibition of insulin secretion (DeDivitiis et al., 1968). Propranolol has also been reported to enhance hypoglycaemia from its ability to interfere with catecholamine-induced glycogenolysis (Hansten, 1979). However, in sulphonylurea treated patients who developed hypoglycaemia, propranolol also prevented the rebound of serum glucose (Hansten, 1979) as it does with insulin hypoglycaemia.

PLAN OF STUDY

PLAN OF STUDY

Sl. No.	Groups Normal/ Diabetic	Drugs Pretreatment 5mg in 2% gum acacia	Drugs Pretreatment Dose(mg/kg) (once a day)	Duration	Time of tolbutamide administration	Time of blood collection
1	2	3	4	5	6	7
1.	Normal	2 % gum acacia	5 ml	one day	-	0, 3, 5, 7, 9, and 11 hours
2.	Normal	-	-	-	0 hour	0, 3, 5, 7, 9, and 11 hours
3.	Normal	Aspirin	40	one day	-	0, 3, 5, 7, 9, and 11 hours
4.	Normal	Aspirin	40	one day	0 hour	0, 3, 5, 7, 9, and 11 hours
5.	Normal	Tramadol	150	one day	-	0, 3, 5, 7, 9, and 11 hours
6.	Normal	Tramadol	150	one day	0 hour	0, 3, 5, 7, 9, and 11 hours
7.	Normal	Tolmetin	20	one day	-	0, 3, 5, 7, 9, and 11 hours
8.	Normal	Tolmetin	20	one day	0 hour	0, 3, 5, 7, 9, and 11 hours
9.	Normal	Propranolol	8	one day	-	0, 3, 5, 7, 9, and 11 hours
10.	Normal	Propranolol	8	One day	0 hour	0, 3, 5, 7, 9, and 11 hours
11.	Normal	Metoprolol	10	one day	-	0, 3, 5, 7, 9, and 11 hours
12.	Normal	Metoprolol	10	one day	0 hour	0, 3, 5, 7, 9, and 11 hours

1	2	3	4	5	6	7
28.	Normal	Aspirin	40	7 days	-	0, 3, 5, 7, 9 and 11 hours
29.	Normal	Aspirin	40	7 days	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
30.	Normal	Tramadol	150	7 dys	-	0, 3, 5, 7, 9 and 11 hours
31.	Normal	Tramadol	150	7 days	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
32.	Normal	Tolmetin	20	7 days	-	0, 3, 5, 7, 9 and 11 hours
33.	Normal	Tolmetin	20	7 days	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
34.	Normal	Propranolol	8	7 days	-	0, 3, 5, 7, 9 and 11 hours
35.	Normal	Propranolol	8	7 days	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
36.	Normal	Nebogrolol	10	7 days	-	0, 3, 5, 7, 9 and 11 hours
37.	Normal	Nebogrolol	10	7 days	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
38.	Normal	Atenolol	6	7 days	-	0, 3, 5, 7, 9 and 11 hours
39.	Normal	Atenolol	6	7 dys	8th day, 0 hr.	0, 3, 5, 7, 9 and 11 hours
40.	Normal	Acebutolol	30	7 days	-	0, 3, 5, 7, 9 and 11 hours
41.	Normal	Acebutolol	30	7 days	8th day, 0 hr.	0, 3, 5, 7, 9, and 11 hours
42.	Normal	-	-	-	(25mg/kg) 0 hr.	0, 3, 5, 7, 9 and 11 hours
43.	Normal	-	-	-	(75mg/kg) 0 hr.	0, 3, 5, 7, 9 and h hours

MATERIAL AND METHODS

MATERIAL AND METHODS

MATERIAL:

In the present investigation, effect of concurrent administration and repeated pre-treatment with some anti-inflammatory and beta-adrenoceptor blocking agents was studied on tolbutamide - induced hypoglycaemia. In order to delineate the mechanism of interaction, serum tolbutamide concentration and tolbutamide half-life was estimated along with blood sugar level.

ANIMALS:

Healthy rabbits of either sex weighing between 1 and 1.6 kg were used in this study. Rabbits were divided into 43 groups of 6 each (as detailed in plan of study) to study drug interaction with tolbutamide. Drugs were administered as a single dose or once daily for 7 days, to see their effect on tolbutamide-induced hypoglycaemia. The rabbits were fasted overnight but with easy access to water. On the following day drugs or drug-combinations under study were administered orally in the morning and blood samples were collected at 0, 3, 5, 7, 9, and 11 hours.

CHEMICALS

For estimation of blood glucose:

1. D-Glucose (GR- Sarabhai M. Chemicals).
2. Benzoic Acid (AR-Merck).
3. Sodium Carbonate anhydrous (Analar-BDH)
4. Tartaric acid (Analar - BDH)
5. Copper sulphate (GR-Sarabhai M. Chemicals).
6. Molybdic acid (AR-Russian).
7. Sodium hydroxide (GR-Sarabhai M. Chemicals).
8. Phosphoric acid (GR- Sarabhai M. Chemicals).
9. Sodium tungstate (Analar - BDH)
10. Sulphuric acid (Analar - BDH).

For estimation of Serum tolbutamide:

11. Amylacetate (LR - Sarabhai M. Chemicals).
12. 2, 4-Dinitrofluorobenzene (1-Fluoro-2, 4, Dinitrobenzene - Puriss A.R. - K.L. England).
13. Hydrochloric acid (GR - Sarabhai M. Chemicals).

Other chemicals:

14. Alloxan monohydrate (LOBA-CHEMIE)
15. Xylene (LR - BDH)

REAGENTS:

I. GLUCOSE ESTIMATION

1. STANDARD SUGAR SOLUTIONS:

Three standard solutions were prepared.

(a) A stock solution of 1 percent glucose was prepared with saturated benzoic acid solution and kept in a regrigerator.

(b) A solution containing 2 mg of glucose in 1 ml (20 ml of stock solution diluted to 100 ml with water) was prepared freshly before use.

(c) Solutions containing 0.05and 0.4 mg of sugar in 2 ml made by dilution of (b) with distilled water. The dilute standards were prepared just before the experiment.

2. ALKALINE COPPER SOLUTION:

40 g of pure anhydrous sodium carbonate was dissolved in about 400 ml of water and was transferred to a flask (1 L capacity). 7.5 g of tartaric acid was added and when the later was dissolved 4.5 g of crystallized copper sulphate was added. It was properly mixed and volume was made upto 1 litre. If the chemicals used are not pure, a sediment of Cuprous oxide may form in the course of one or two weeks. If this happens, the clear supernatent reagent was removed with a siphon, or filtered through a good quality filter paper. The reagent can be kept indefinitely.

PHOSPHOMOLYBDIC ACID SOLUTION:

To 35 g of molybdic acid and 5 g of sodium tungstate, 200 ml of 10 % sodium hydroxide and 200 ml

of water were added. It was boiled vigorously for 20-40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Then it was cooled, diluted to about 360 ml and to it 125 ml of concentrated (85 %) phosphoric acid was added. The final volume was made upto 500 ml with distilled water.

SODIUM TUNGSTATE SOLUTION:

10 gm of sodium tungstate (Analar - BDH) was dissolved in 100 ml of distilled water and kept in glass stoppered bottle.

STANDARD TOLBUTAMIDE (400 Mg/ml)

40 mg tolbutamide I.P. was dissolved in 10 ml of amyl acetate. From this concentrated (4000 µg/ml) tolbutamide solution in final standard solution was prepared by diluting 1 ml of concentrated standard with 9 ml of amyl acetate. This solution contains 400 µg tolbutamide per ml . It was stored in refrigerator.

AMYL ACETATE:

Amyl acetate is shaken with the same volume of water (Distilled water) and finally preserved over distilled water.

DNFB REAGENT:

0.1 ml of 2, 4 -Dinitrofluorobenzene(DNFB) was dissolved in 100 ml of amyl acetate and stored in refrigerator.

HYDROCHLORIC ACID:

0.1 N Hydrochloric acid solution was prepared in distilled water and stored in glass stoppered bottle.

ALLOXAN MONOHYDRATE SOLUTION:

Fresh solution of 155 mg/ml of alloxan monohydrate was prepared in distilled water just before use.

DRUGS:

Anti-inflammatory drugs under study are not soluble in distilled water but beta-adrenergic blockers are soluble. To maintain the homogeneity, all the following drugs were prepared in 2 % gum acacia.

1. Acebutolol (30 mg/kg).
2. Aspirin (acetyl salicylic acid) IP (Vikash Pharma, Bombay).
3. Atenolol (CIBA - Bombay).
4. Metoprolol tartrate.
5. Propranolol (ACC I - Madras)

6. Tolbutamide IP (Hoechst - Bombay).

7. Tolmetin (MC Neil Laboratories - Washington).

8. Tromeril (Unichem - Bombay).

Vehicle:

2% Gum acacia IP (Vikash Pharma - Bombay).

METHODS:

COLLECTION OF BLOOD

The marginal ear vein was selected for collection of blood in rabbits. Xylene was not used to make the vessels prominent because it caused haemolysis and affected collection of serum in preliminary experiments. Therefore blood vessels were made prominent by applying heat with the help of an electric lamp to the pinna of the rabbit. Then a cut was made with the help of sharp edged blade, on marginal ear vein. Blood was collected in two different vials (i) Fluoride vials (for blood sugar), (ii) plain well dried vials(for serum tolbutamide).

ESTIMATION OF BLOOD GLUCOSE:

Blood glucose was estimated by Folin & Wu(1920) method.

PRINCIPLE:

When the protein-free blood filterate is heated with alkaline copper solution, cuprous oxide is formed (glucose reduces cupric oxide to cuprous oxide). Cuprous oxide thus formed when treated with phosphomolybdic acid solution forms a blue colour which is compared with that of a standard with the help of colorimeter.

PROCEDURE:

3.5 ml of distilled water was taken in a centrifuge tube and to it 0.1 ml of blood was added. 0.2 ml of 10% sodium tungstate and 0.2 ml of 0.67N Sulphuric acid were added subsequently to precipitate the blood proteins. After mixing vigorously it was allowed to settle for sometime and then centrifuged for 10 minutes at 1,000 r.p.m. 2 ml of supernatant fluid was pippetted into a Folin's sugar tube. If blood sugar levels are expected to be too high the supernatant was diluted with same amount of distilled water. 2 ml of distilled water (blank) and 2 ml of standard sugar solution containing 0.05and 0.1 mg of glucose (standards) were taken in similar tubes. 2 ml of the alkaline copper solution was added. Then the tubes were kept in a boiling water bath for 3 minutes and then cooled in running water without shaking. Then to each tube 2 ml of phosphomolybdic acid

reagent was added. After about 1 minute distilled water was added to the mark (12.5 ml) and mixed thoroughly. It is essential that adequate attention be given to this mixing because the greater part of the blue colour is formed in the bulb of the tube. Since the colour is not stable for long time the colorimetric readings were taken in 30 minutes. The optical density (O.D.) was determined at 420 mμ setting the photometer to 100 % transmittance with the blank.

CALCULATION

$$\frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \text{glucose (mg) in standard} \times \frac{100}{0.05}$$

= Blood glucose in mg per 100 ml .

ESTIMATION OF SERUM TOLBUTAMIDE

Serum tolbutamide was estimated by the method of Spingler (1967).

PRINCIPLE

Serum tolbutamide is dissolved in amyl acetate (forms a distinct separate layer on serum) which is separated with the help of centrifuge. 2.4-Dinitro-fluorobenzene forms yellow colour after reacting with tolbutamide which is estimated colorimetriegily at 390 mμ.

PROCEDURE

1 ml of clear serum was shaken with 5 ml of amyl acetate for one minute in an ordinary test tube. Then 0.2 ml 1 N hydrochloric acid was added and shaken thoroughly for 3 minutes and thentransferred to a centrifuge tube. After centrifuging for 2 minutes at 1000 r.p.m., 4 ml of the clear supernatant amyl acetate solution was pippetted into a graduated test tube. 1 ml DNFB (2.4-dinitrofluorobenzene) reagent solution was added. After mixing well, the test tube was placed in an oil bath maintained at 150 ± 1°C(ground nut oil was used to make oil bath) and left for 5 minutes. Then it was cooled in a cold water bath at room temperature. For preparing the blank, 1 ml of distilled water and for standard, 1 ml standard tolbutamide solution were used instead of serum. The O.D. of standard and samples were measured in an Elico Spectro photometer at 380 mμ by setting the instrument at 100% transmission with the blank.

CALCULATION

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 400 = \text{Serum tolbutamide in } \mu g/ml .$$

INDUCTION OF DIABETES BY ALLOXAN

Alloxan monohydrate ($C_4H_2N_2O_4$, H_2O) was used to produce experimental diabetes in rabbits. Ten healthy rabbits of either sex weighing between 1 and 1.5 kg were selected and kept on fast overnight.

In the next morning, a fresh solution of alloxan monohydrate (155 mg/ml) was prepared in distilled water. Alloxan solution was injected into the marginal ear vein at a dose of 1ml/kg. Severe hypoglycaemia occurs within 1 to 4 hours of alloxan injection (causing convulsions and death), which may last upto 48 hours (Rerup, 1970), 5 gm of glucose was given 4 hourly with the help of feeding cannula to every alloxan treated rabbit.

METHOD OF DETERMINATION OF SERUM HALF-LIFE OF TOLBUTAMIDE:

The biological half-life (t ½) is defined as the time required for blood level to decrease from the peak level by 50%(Show and Beaser, 1971). Serum tolbutamide concentration versus time was plotted on semilogarithmic scale. The plasma t ½ was determined by interpolating the 50% of plasma peak level.

STATISTICAL ANALYSIS :

The data obtained in the study were analysed by Student's 't' test. The per se effects of tolbutamide and other drugs under study for interaction were compared against the effect of the treatment with the vehicle (2 % gum acacia) whereas the effect of combinations were compared against tolbutamide.

OBSERVATIONS AND RESULTS

RESULTS AND OBSERVATIONS

In the present study the effects of concurrent administration as well as repeated pre-treatment with anti-inflammatory and beta-adrenoceptor blocking agents on tolbutamide induced hypoglycaemic response and serum tolbutamide concentration and its plasma half-life were studied. Among the anti-inflammatory agents acetylsalicylic acid (aspirin), the most well studied nonsteroidal anti-inflammatory drug; tromexil and tolmetin comparatively recent and newly introduced anti-inflammatory drugs were selected for interaction study. Similarly propranolol the oldest, potent and most clinically used beta-adrenoceptor blocking agent, and some new and cardioselective beta-blocking agents like metoprolol, atenolol and acebutolol were chosen amongst a vast number of beta-blockers.

Tolbutamide and anti-inflammatory agents selected for interaction study were administered orally as a suspension in 2 % gum acacia, since they are not soluble in water. But the beta-blockers although soluble in water were administered orally prepared in 2 % gum acacia to maintain homogeneity of the vehicle. In control experiments 2 % gum acacia was used to see the effect of only vehicle on blood sugar. The drugs were administered at 8 A.M. and

Table. 5 : Effect of tolbutamide (graded doses) on blood sugar level in normal and diabetic rabbits.

Type of animal	Dose of tolbutamide (in 2 % gum acacia	Blood sugar change in % Mean ± S.E.					
		0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Normal	Control (2 % gum acacia)	100	98.79 ±1.01	100.1 ±0.6	101.22 ± 1.58	101.98 ± 1.42	102.49 ± 1.02
	25 mg/kg	100	89.86** ±1.68	72.96*** ±1.98	91.15** ±1.29	99.54 ±2.38	101.89 ± 2.01
	50 mg/kg	100	80.85*** ±1.55	68.88*** ± 0.98	85.45*** ±2.01	98.54 ±1.62	102.31 ±2.41
	75 mg/kg	100	71.16*** ±2.04	51.15*** ±1.88	72.37*** ±2.17	89.64** ±1.35	100.52 ± 2.00
Diabetic	Control (2 % gum acacia)	100	98.78 ±1.6	102.1 ± 2.01	100.7 ± 2.27	99.38 ±1.95	98.25 ±3.00
	Tolbutamide (50 mg/kg)	100	68.2*** ±2.05	72.41*** ±1.75	95.94 ±2.28	101.48 ±1.35	103.44 ±2.12

*, **, *** indicate 't' values < 0.05, < 0.01, $< .001$ respectively.

EFFECT OF TOLBUTAMIDE ON RABBIT BLOOD SUGAR.

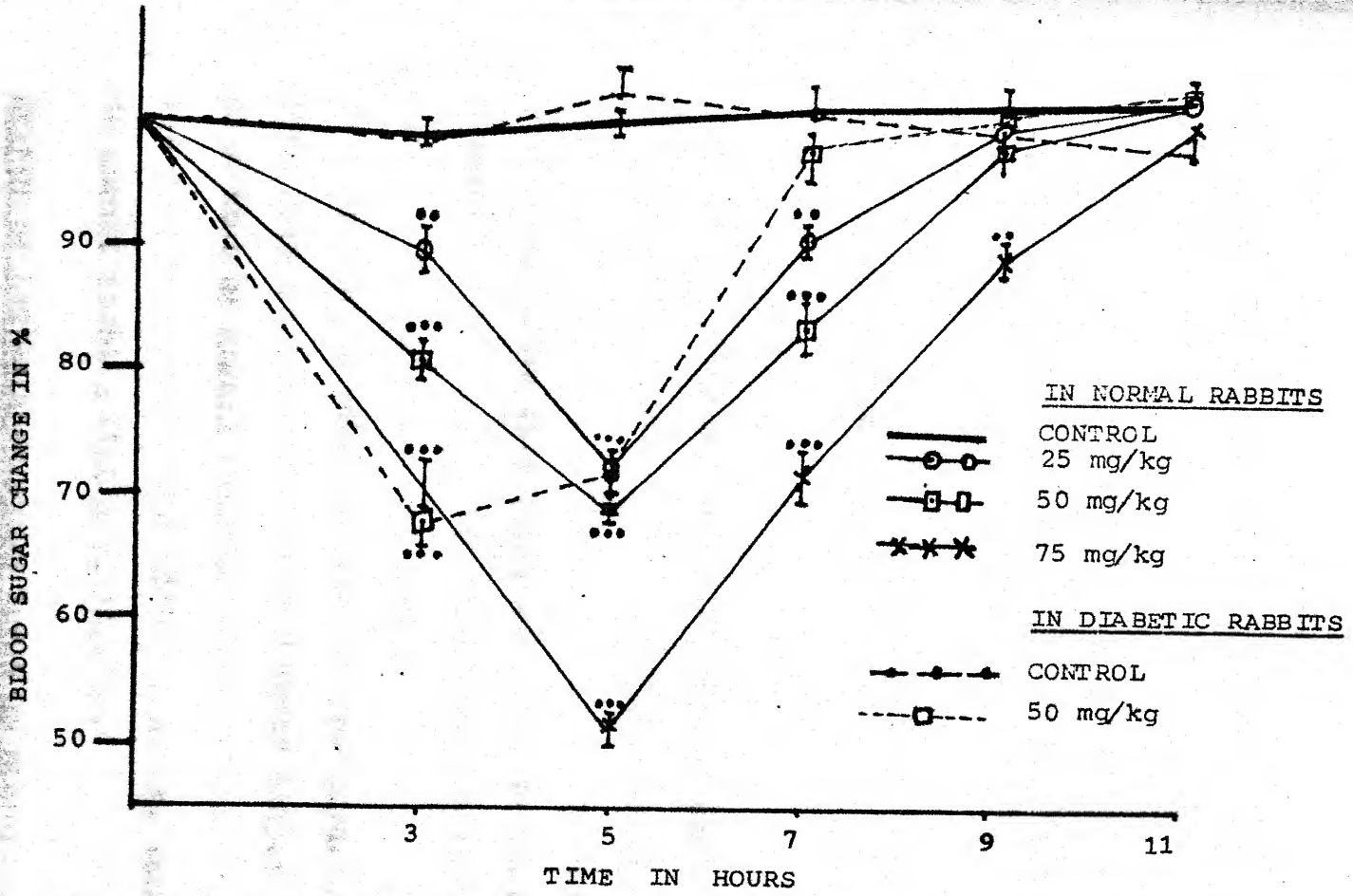


Fig. 2 : Shows effect of graded doses of tolbutamide on blood sugar level in normal and diabetic rabbits. Peak hypoglycaemic response is observed at 5 hours in normal and at 3 hours in diabetic rabbits (alloxan, 155 mg/kg I.V.). •• , ••• indicate P values < 0.01 and < 0.001 respectively.

blood sugar and serum tolbutamide concentration were measured from 8 A.M. to 7 P.M. . In chronic treatment groups the drugs were administered daily at 1 P.M. for 7 consecutive days. The time od drug administration and measurement of blood sugar and serum tolbutamide level was kept constant to avoid variations due to circadian effect

EFFECT OF TOLBUTAMIDE ON BLOOD SUGAR OF RABBITS

Following oral administration of 2 % gum acacia the blood sugar level over 11 hours of study was not significantly affected. Tolbutamide produced a dose dependant hypoglycaemia. The hypoglycaemic response reached a peak level after five hours and completerecovery was observed after 9 hours. For subsequent interaction studies tolbutamide was used at a dose of 50 mg/kg. In diabetic rabbits also tolbutamide produced hypoglycaemia but the peak response was observed at 3 hours comparatively earlier than that of normal rabbits. However, the blood sugar level returned to control value within 9 hours, almost similar to normal rabbits (Table 5, Fig. 2).

EFFECT OF ANTI-INFLAMMATORY DRUGS ON BLOOD SUGAR OF NORMAL RABBITS :

Aspirin at a dose of 40 mg/kg produced a marked hypoglycaemia with a peak effect at 5 hours and the effect persisted beyond 9 hours.

TABLE. 6 : EFFECT OF ANTI-INFLAMMATORY AGENTS AND ANTIANGINAL BETA-BLOCKERS (SINGLE DOSE) ON BLOOD SUGAR LEVEL IN NORMAL RABBITS.

DRUGS (mg/kg in ± 2 % gum acacia)	BLOOD SUGAR CHANGE (in %) MEAN ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
2 % gum acacia (5ml)	100	98.79 ±1.01	100.1 ± 0.6	101.22 ± 1.58	101.98 ± 1.42	102.49 ± 1.02
Aspirin(40)	100	84.59** ±2.05	85.77*** ± 1.44	91.85* ± 3.1	96.89 ± 2.64	103.11 ± .25
Tremacil (150)	100	102.3 ± 2.64	102.31 ± 1.7	98.35 ±2.17	103.31 ± 3	102.64 ± 1.26
Tolbutin (20)	100	84.53** ±3.08	81.55*** ± 1.14	90.1* ± 2.39	96.73 ± 1.86	100 ± 1.21
Propranolol(8)	100	94.95 ±1.18	90.68** ± 1.46	94.35 ± 2.46	96.1* ± 1.4	98.08 ± 2.66
Metoprolol(10)	100	100.19 ±1.8	97.75 ± 1.38	98.86 ± 1.86	96.95 ± 2.68	98.76 ± 1.49
Atenolol (6)	100	98.19 ±0.21	94.38** ± 1.1	96.5 ± 1.88	98.45 ±1.93	100.78 ±1.95
Acebutolol(30)	100	97.46 ±2.03	100.55 ± 1.26	100.61 ± 1.1	100.31 ± 1.95	102.17 ± 2.3

*, **, *** indicate p values < 0.05, < 0.01 and $< .001$ respectively.

Note :- 0, 3, 5, 7, 9 and 11 hour indicate 8 A.M., 11 A.M., 1 P.M., 3 P.M., 5 P.M. and 7 P.M. of the day of experiment. Drugs are administered at 8 A.M.

TABLE. 7: EFFECTS OF REPEATED TREATMENT (7 days) OF ANTI-INFLAMMATORY AGENTS AND BETA-ADRENERGIC BLOCKERS ON BLOOD SUGAR OF NORMAL RABBITS.

DRUGS (mg/kg in 2% gum acacia	Blood sugar on first day	Blood sugar change (in %) on 8th day (Mean ± S.E.)					
		0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
2 % gum acacia(5 ml)	100	100.06±1.86	98.61±0.9	99.05 ± 1.1	102.36 ± 1.46	101.54 ±1.3	103.1±1.13
Aspirin (40)	100	88.22±1.5**	89.33±2.07**	93.33± 2.04	95.98 ± 2.11	96.69 ± 2.31	98.3±2.05
Tramadol (150)	100	98.39±1.28	101.36±2.01	98.98± 1.72	97.0 ± 2.01	98.55± 1.74	101.85±2.24
Nalbuphin (20)	100	90.67±2.1*	91.72±1.22**	96.12±1.76	98.34±1.92	96.31± 2.66	99.19±2.83
Propranolol (9)	100	94.28±3.4	95.38±2.48	95.2 ±3.18	98.28±2.25	100.14±2.8	98.51±1.8
Metoprolol(10)	100	98.11±3.09	95.14±2.81	95.74±1.86	97.2 ±2.9	98.44±3.1	98.35±2.15
Atenolol (6)	100	94.33±1.45	95.0 ±2.8	95.91±2.3	96.81±1.61	97.0 ±2.1	100.8±3.06
Acebutolol(30)	100	97.54±1.55	96.4 ±2.4	95.82±2.6	96.3 ±2.72	100.0±2.85	99.9±3.15

*, ** indicate / ? values < 0.05 and < 0.01 respectively

Notes- 0, 3, 5, 7, 9 and 11 hour indicate 8 A.M., 11 A.M., 1 P.M., 3 P.M., 5 P.M. and 7 P.M. respectively on 8th day. Drugs are administered daily at 8A.M. for 7 consecutive days.

EFFECT OF ANTI-INFLAMMATORY AGENTS ON BLOOD SUGAR LEVEL IN NORMAL RABBITS.

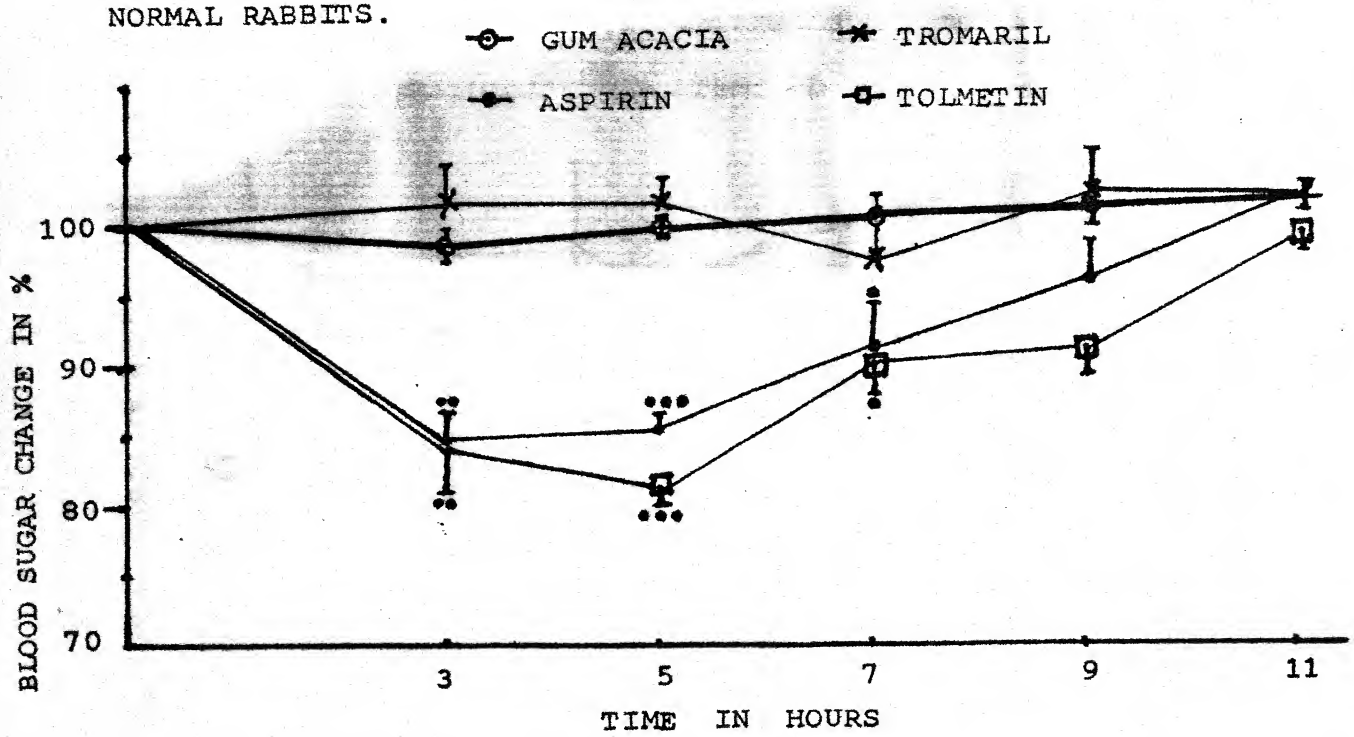


Fig. 3 : Shows effect of aspirin (40 mg/kg), tromaril (150 mg/kg) and tolmetin (20 mg/kg) on blood sugar level in normal rabbits after single dose administration. Aspirin and tolmetin show significant hypoglycaemic response. •, ••, ••• indicate P values < 0.05, < 0.01 < 0.001 respectively.

EFFECT OF BETA-BLOCKERS ON BLOOD SUGAR IN NORMAL RABBITS

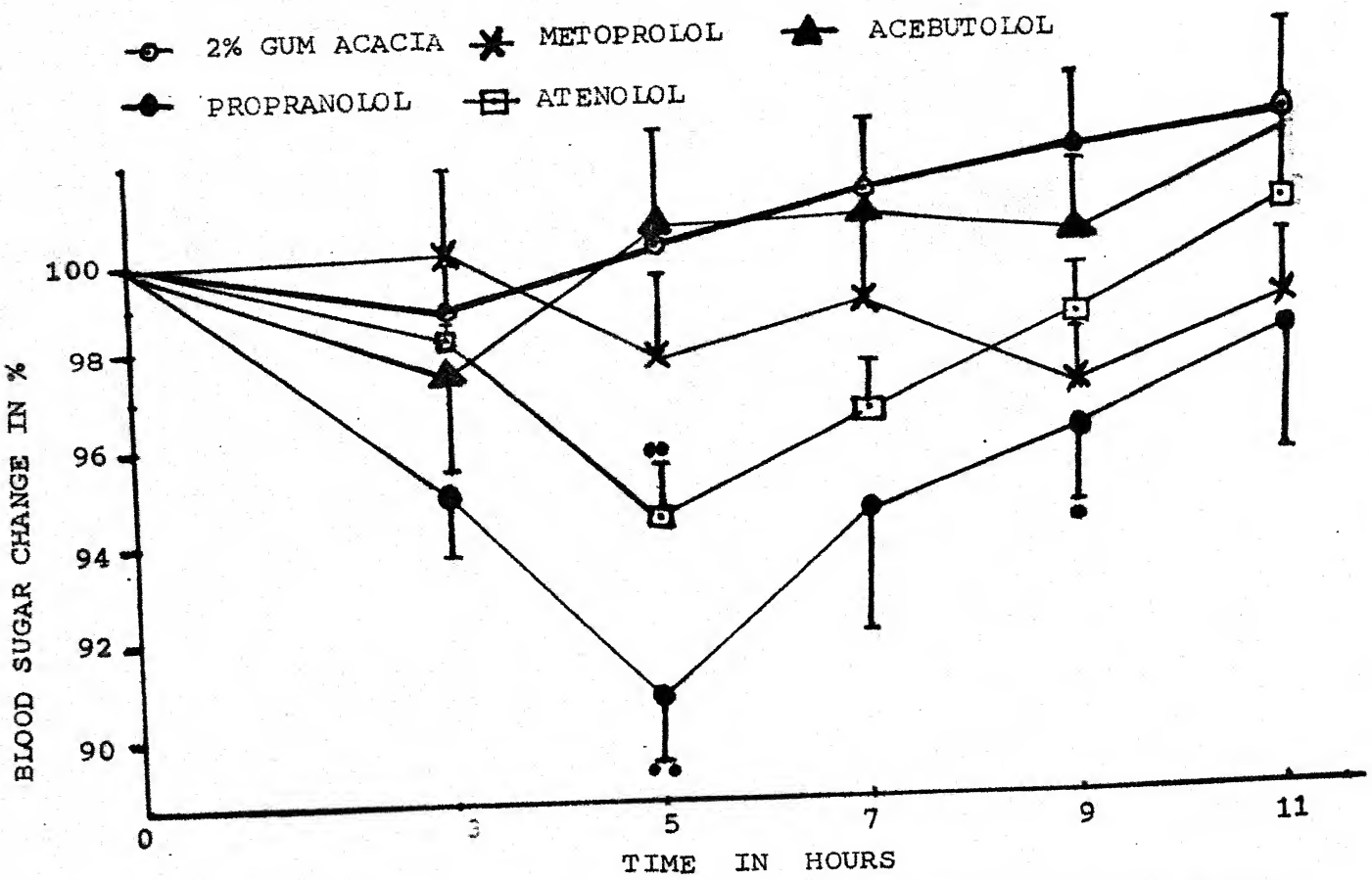


Fig. 4 : Shows effect of propranolol (8 mg/kg), metoprolol (10 mg/kg), atenolol (6 mg/kg) and acebutolol (30 mg/kg) on blood sugar level in normal rabbits after single dose administration. Propranolol and atenolol show significant hypoglycaemia. •, •• indicate P values < 0.05 and < 0.01 respectively.

EFFECT OF REPEATED ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON BLOOD SUGAR LEVEL IN NORMAL RABBITS.

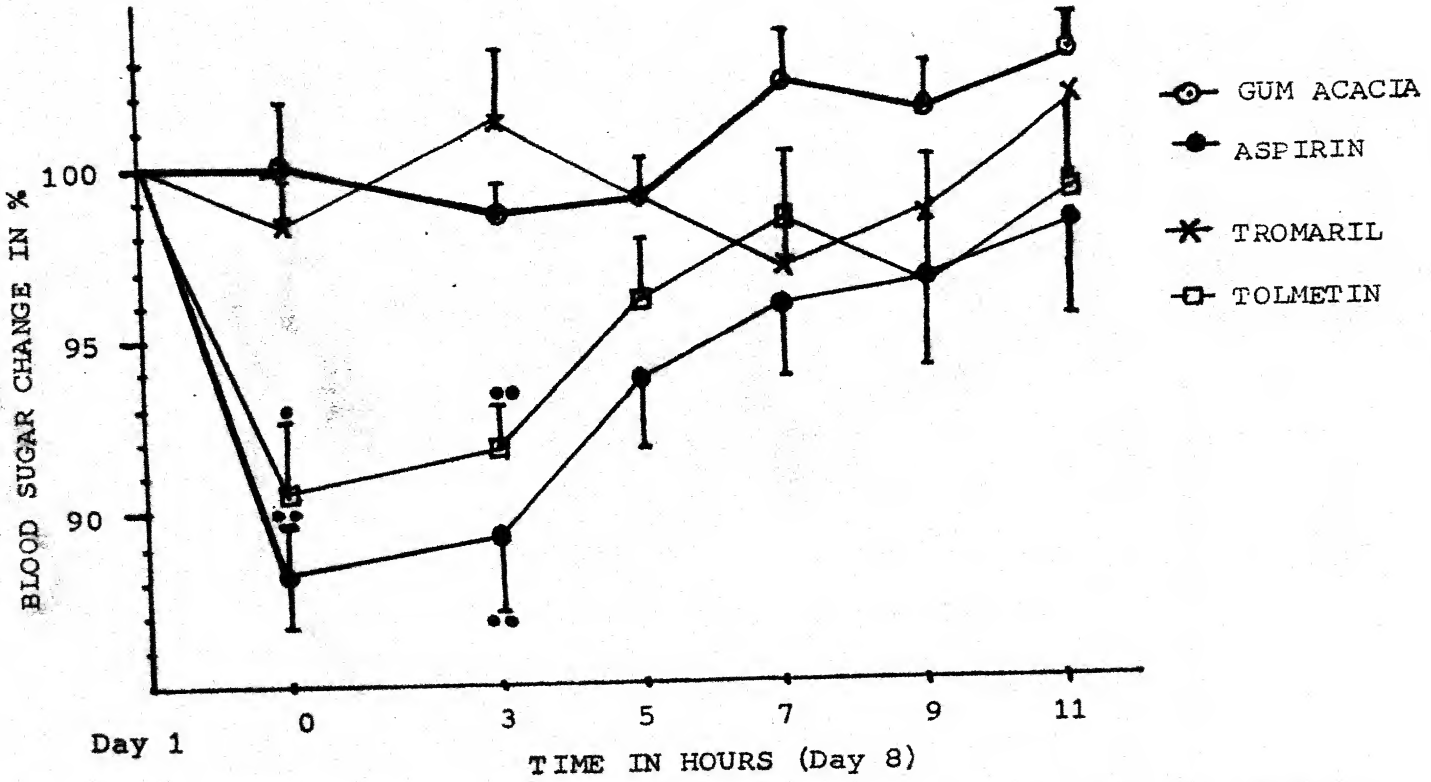


Fig. 5 : Shows effect of anti-inflammatory agents on blood sugar level in normal rabbits after daily oral treatment for 7 days. Blood sugar level is recorded from 8 A.M. to 7 P.M. on the 8th day without drug administration. The hypoglycaemic response of aspirin and tolmetin persists upto 7 P.M. •, •• indicate P values < 0.01 and < 0.001 respectively.

EFFECT OF BETA-BLOCKERS ON BLOOD SUGAR LEVEL OF THE RABBITS

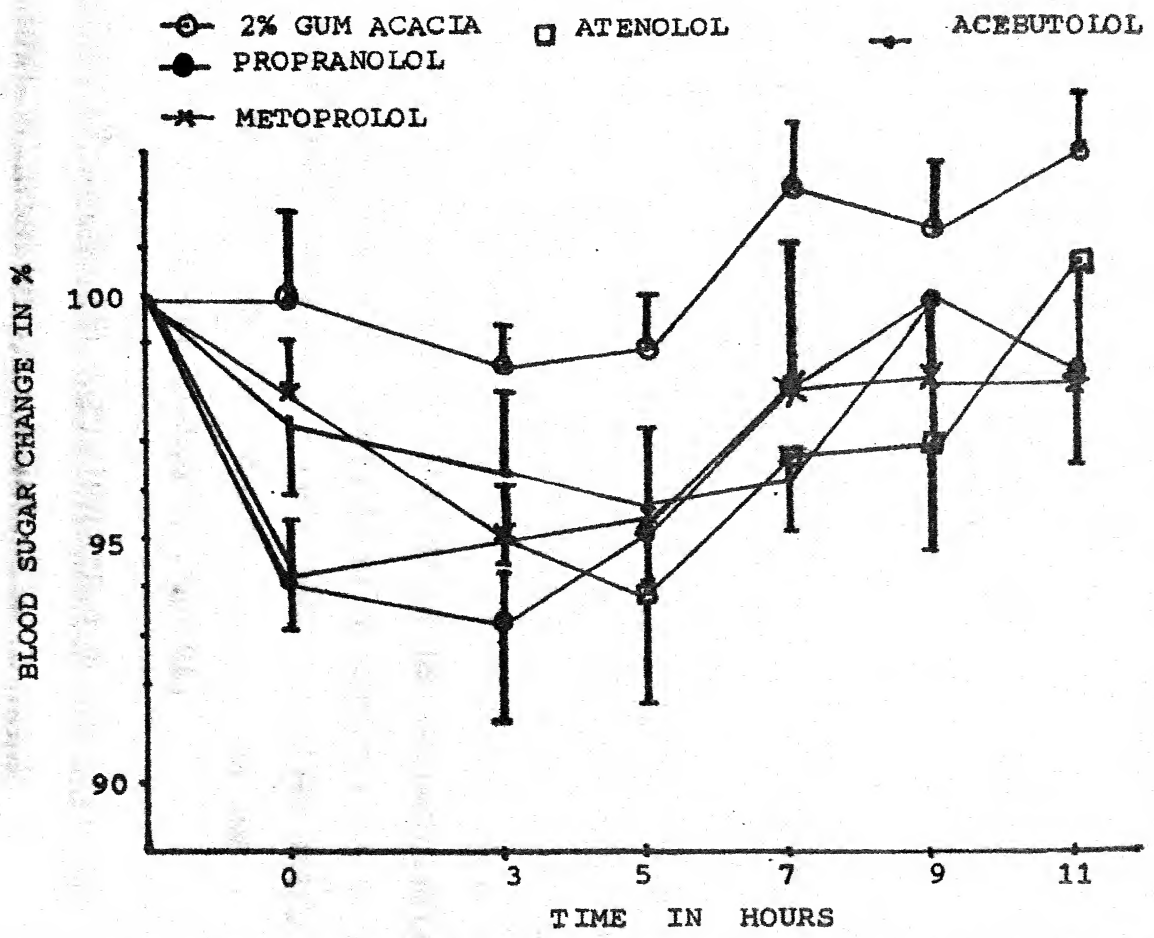


Fig. 6 : Shows effect of beta-blockers on blood sugar level in normal rabbits after daily oral treatment for 7 consecutive days. Blood sugar level was recorded on 8th day from 8 A.M. to 7 P.M. without drug administration. Beta-blockers do not show any persistent hypoglycaemia on 8th day.

Tolmetin at a dose of 20 mg/kg also exhibited a significant hypoglycaemic response with a peak effect at 5 hours and complete recovery was attained at 11 hours. However tromaril at a dose of 160 mg/kg did not show any effect on blood sugar level (Table - 6, Fig. - 3).

Aspirin and tolmetin were administered daily orally for 7 days. On the 8th day without the drug administration hypoglycaemic effect persisted significantly upto 3 hours. But tromaril did not show any such effect (Table-7 Fig.-6).

EFFECT OF BETA-ADRENERGIC BLOCKERS ON BLOOD SUGAR OF NORMAL RABBITS

Propranolol (8 mg/kg) and atenolol (6 mg/kg) produced a slight but significant lowering of blood sugar level with a peak hypoglycaemia at 5 hours and the effect almost reversed after 11 hours. However, the other two beta-blockers metoprolol (10 mg/kg) and acebutolol(30 mg/kg) did not influence the blood sugar concentration to any extent (Table 6, Fig. 4). Beta blockers after daily treatment for 7 days did not not show any effect or blood sugar on the 8th day (Table 7, Fig. 6).

EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON TOLBUTAMIDE INDUCED HYPOGLYCAEMIA

(a) Single dose effect

Concurrent administration of aspirin (40 mg/kg) and tolbutamide (80 mg/kg) increased the hypoglycaemia induced by tolbutamide alone. The potentiation of hypoglycaemia by aspirin was however, significant at 3, 5, and 7,

TABLE - 8 : EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS (SINGLE DOSE) ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

Drug (mg/kg in 2 % gum acacia	Blood sugar change (in %) Mean ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Tolbutamide (50)	100	80.83 ±3.53	68.88 ± .98	83.45 ±2.01	98.58 ±1.62	102.31 ± 2.41
Tolbutamide (50) + Aspirin (40)	100	73.31* ±3.15	60.85** ±1.42	74.75* ±1.47	91.53 ±4.09	100.67 ± 1.77
Tolbutamide (50) + Brucanil (150)	100	78.61 ±3.22	70.05 ±2.92	82.81 ±2.03	98.27 ±5.44	103.32 ± 4.64
Tolbutamide (50) + Tolmetin (20)	100	74.86* ±1.39	64.88* ±3.05	75.92* ±3.26	94.7 ±1.95	98.15 ±3.25

*, ** indicate P values < 0.05 and < 0.01 respectively.

TABLE 9 : EFFECT OF REPEATED ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

Drug (mg/kg in 2% gum acacia)	Blood sugar change (in %) Mean ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Tolbutamide (50)	100	80.83 ±1.53	68.88 ± .98	83.45 ±2.01	98.56 ±1.62	102.31 ±2.41
Aspirin (40) + Tolbutamide (50)	100	75.17* ±1.19	68.61* ±1.29	76.25* ±1.79	99.00 ±2.18	102.92 ±2.47
Tramal (150) + Tolbutamide (50)	100	79.89 ±1.9	70.47 ±2.2	93.00 * ±2.87	101.56 ± 1.46	98.5 ± 2.66
Voltarin (20) + Tolbutamide (50)	100	78.38* ±1.16	61.63** ±1.02	87.22 ±2.06	100.31 ± 2.55	100.11 ±1.04

*, ** indicate P values < 0.05 and < 0.01 respectively.

EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS (SINGLE DOSE) ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

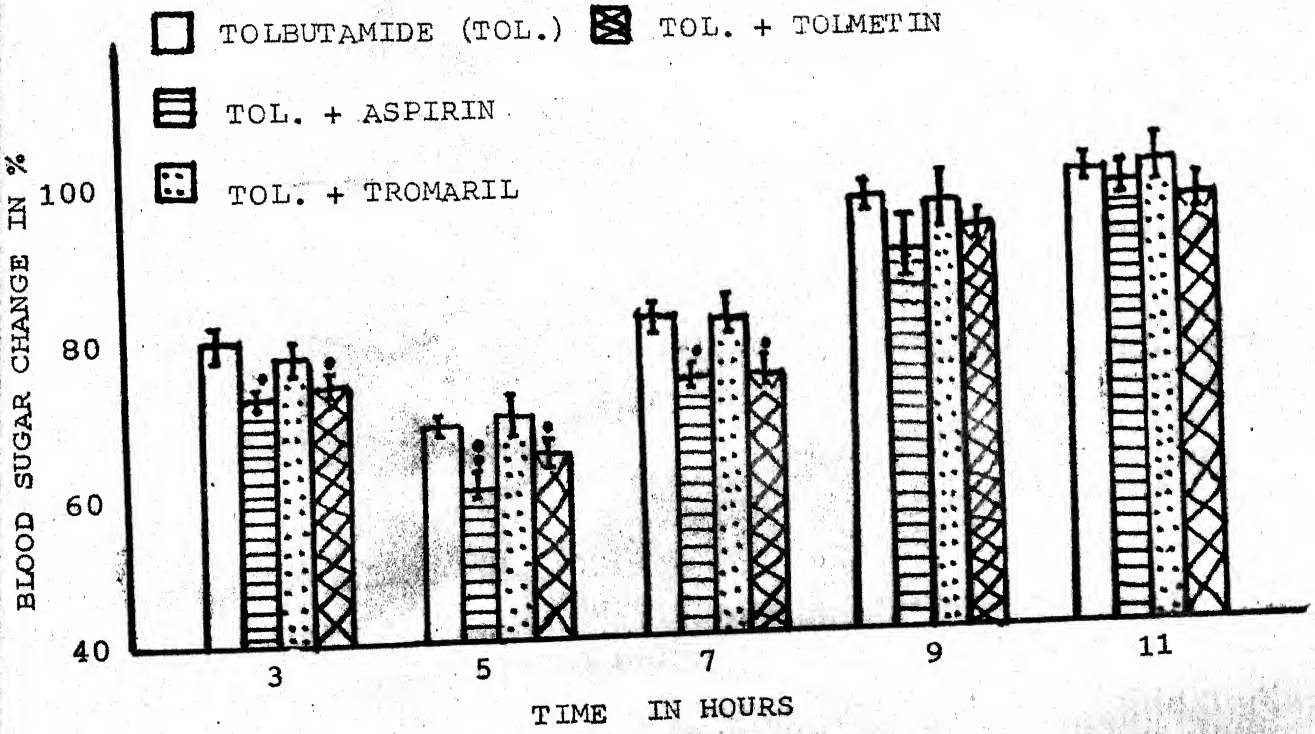


Fig. 7 : Shows effect of anti-inflammatory drugs on tolbutamide (50 mg/kg) induced hypoglycaemia in normal rabbits. Tolmetin and aspirin show potentiation. •, •• indicate P values < 0.05 and < 0.01 respectively.

EFFECT OF REPEATED ADMINISTRATION (7 DAYS) OF ANTI-INFLAMMATORY AGENTS ON TOLBUTAMIDE (T) HYPOGLYCAEMIA IN NORMAL RABBITS.

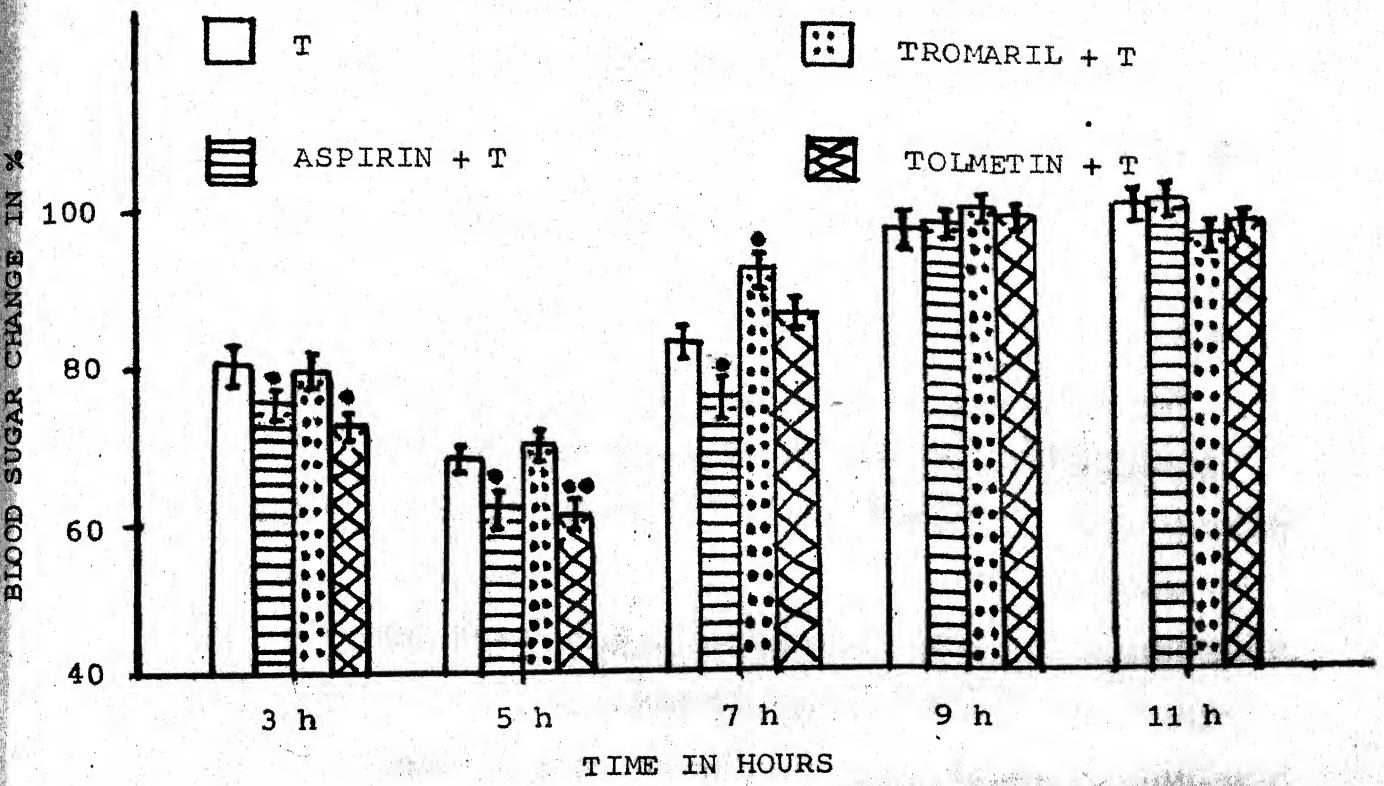


Fig. : 8 - Shows effect of repeated administration (7 days) of anti-inflammatory drugs on Tolbutamide (T) induced hypoglycaemia in normal rabbits. Aspirin and Tolmetin produce significant potentiation. •, •• indicate P values < 0.05 and < 0.01 respectively.

hours of administration. Hypoglycaemia produced by the combination of tromaril (100 mg/kg) and tolbutamide (80 mg/kg) was almost equal to the hypoglycaemia produced by tolbutamide (80 mg/kg) alone. Tolmetin potentiated the hypoglycaemic response of tolbutamide. The potentiation was significantly observed upto 7 hours only(Table 8, Fig.7).

(b) Effect of repeated administration

Pre-treatment with aspirin (40 mg/kg) for 7 days increased significantly the hypoglycaemic response of tolbutamide than that of untreated rabbits. Signigicant change was seen at 3, 5 and 7 hours. Quantitatively similar potentiation of hypoglycaemia was noted with tolmetin (20 mg/kg/day for 7 days). However, it was only significant at 3 and 5 hours. But treatment with tromaril for 7 days did not affect tolbutamide-hypoglycaemia to any significant extent (Table 9, Fig. 8). However, recovery of hypoglycaemic response was comparatively earlier with tromaril.

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-BLOCKERS ON TOLBUTAMIDE-INDUCED HYPOGLYCAEMIA

(a) Single dose effects

Propranolol (5 mg/kg) and atenolol(6 mg/kg) slightly increased the hypoglycaemic effect of tolbutamide when administered concurrently. In addition, they also prolonged the hypoglycaemic response as blood sugar level

TABLE. 10 : EFFECT OF CONCURRENT ADMINISTRATION OF BETA-ADRENERGIC BLOCKERS (SINGLE DOSE) ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

Drug (mg/kg in 2 % gum acacia)	Blood sugar change (in %) Mean ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Tolbutamide (50)	100	80.03 ±1.53	68.89 ±0.72	83.45 ±2.01	98.58 ±1.62	102.31 ± 2.41
Propranolol (20) + Tolbutamide (50)	100	75.38 ±2.26	62.22* ±2.35	75.87* ±0.81	87.2** ±2.28	95.81 ± 1.09
Metoprolol (10) + Tolbutamide (50)	100	78.77 ±2.19	69.28 ±3.55	78.15 ±1.78	95.11 ±2.77	101.52 ± 2.5
Atenolol (6) + Tolbutamide (50)	100	76.17 ±2.93	66.19 ±2.86	76.48* ±1.07	91.03* ±1.49	98.32 ±2.39
Acebutolol(30) + Tolbutamide (50)	100	77.22 ±1.86	70.76 ±2.45	82.9 ±2.98	90.94* ±2.01	100.88 ±3.79

*, ** indicate P values < 0.05 and < 0.01 respectively.

TABLE 11 : EFFECT OF REPEATED ADMINISTRATION (for 7 days) OF BETA-ADRENERGIC BLOCKERS ON TOLBUTAMIDE REDUCED HYPOGLYCAEMIA IN NORMAL RABBITS

Drugs (mg/kg in 2 % gum acacia)	Blood sugar change (in %) Mean ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Tolbutamide (50)	100	80.83 ±1.53	68.88 ±1.98	83.45 ±2.01	91.58 ±1.62	102.31 ± 2.41
Propranolol (8) + Tolbutamide (50)	100	74.76* ±1.36	66.54 ±2.01	74.00* ±2.29	87.86** ±2.03	96.95 ±1.99
Metoprolol (10) + Tolbutamide (50)	100	79.79 ±1.35	75.27 ±2.27	87.89 ±2.4	94.67 ±1.86	99.36 ±1.64
Atenolol (6) + Tolbutamide (50)	100	81.87 ±2.44	74.35 ±2.46	76.62* ±1.13	90.35* ±1.81	100.39 ±1.28
Acebutolol (30) + TOLBUTAMIDE (50)	100	79.26 ±2.04	73.15 ±1.53	86.28 ±1.00	94.7 ±2.77	102.99 ±3.6

*, ** indicate P values < 0.05 and < 0.01 respectively.

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-BLOCKERS (SINGLE DOSE) ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

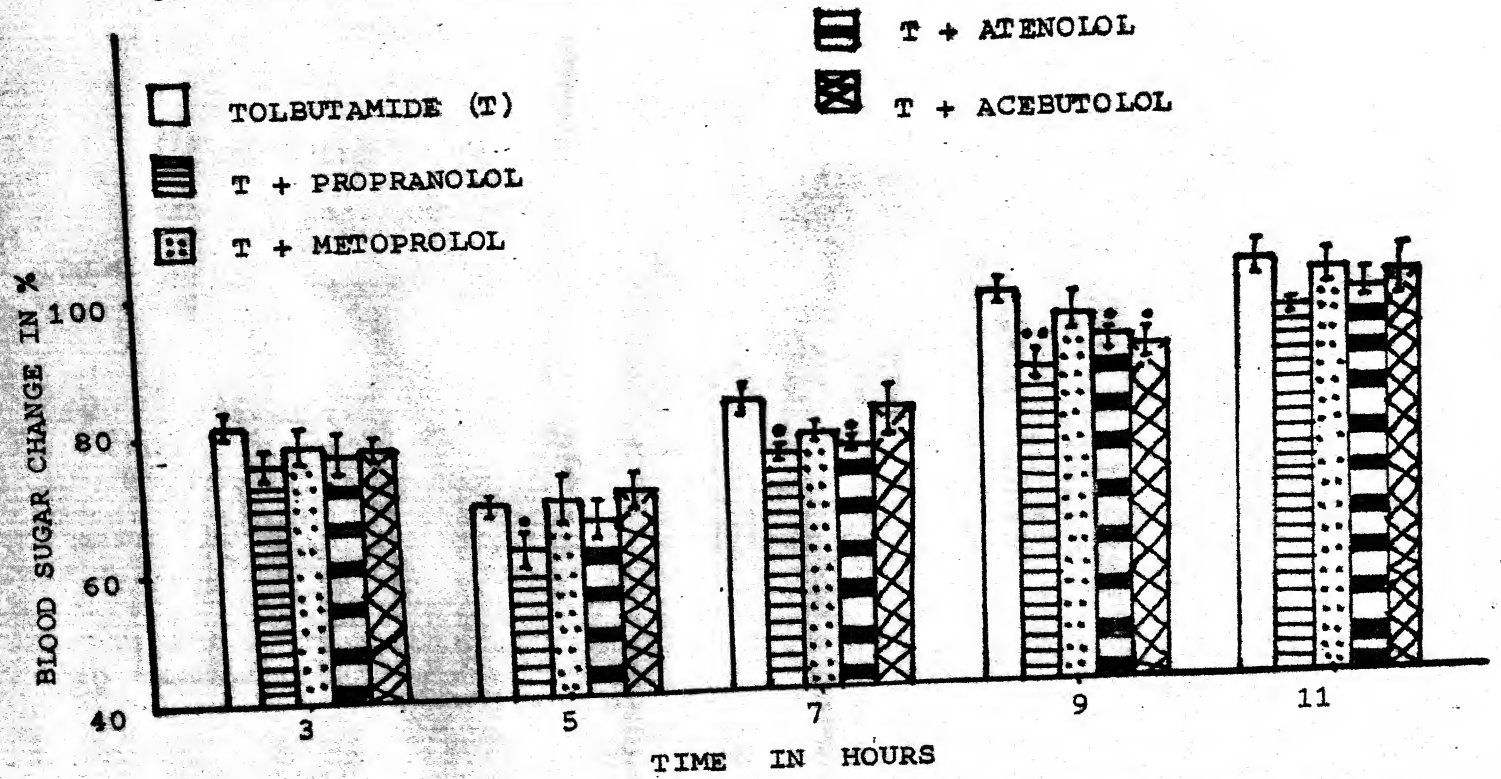


Fig. 9 : Shows effect of concurrent administration of beta-blockers on Tolbutamide (50 mg/kg) induced hypoglycaemia in normal rabbits. Propranolol and atenolol show potentiation. •, •• indicate P values < 0.05 and < 0.01 respectively.

EFFECT OF REPEATED ADMINISTRATION (7 DAYS) OF BETA-BLOCKERS ON TOLBUTAMIDE HYPOGLYCAEMIA IN NORMAL RABBITS.

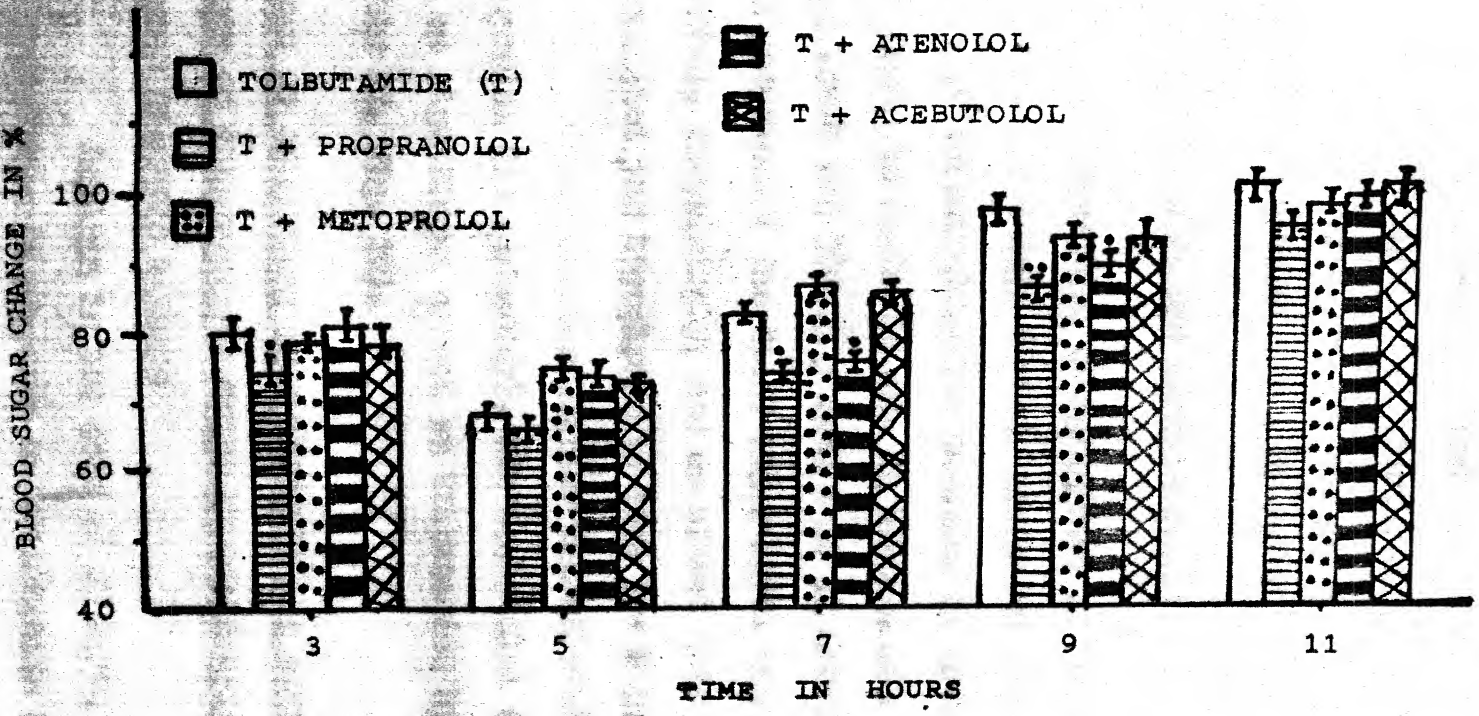


Fig. 10 : Shows effect of repeated administration (7 days) of beta-blockers on tolbutamide-induced hypoglycaemia in normal rabbits. Propranolol and atenolol show potentiation. •, •• indicate P values < 0.05 and < 0.01 respectively.

did not return to normal level even upto 11 hours. But metoprolol (10 mg/kg) and acebutolol (30 mg/kg) neither increased the hypoglycaemia nor prolonged the hypoglycaemic effect of tolbutamide (50 mg/kg).(Table 10, Fig. 9).

(b) Effect of repeated administration

Propranolol (5 mg/kg/day) after repeated treatment for 7 days further increased the hypoglycaemic effect of tolbutamide. The duration of hypoglycaemia was also found to be increased. In tolbutamide group blood sugar level was 103.31 ± 2.41 % at 11 hours whereas with propranolol the blood sugar level was on 69.96 ± 1.30% Atenolol, surprisingly, had no effect upto 6 hours, but potentiated the hypoglycaemia from 7 - 9 hours.

Other cardioselective beta-blocking agents metoprolol (10 mg/kg/day), and acebutolol (30 mg/kg/day) had no significant effect on tolbutamide hypoglycaemia (Table 11, Fig. 10).

EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON TOLBUTAMIDE-INDUCED HYPOGLYCAEMIA IN ALLOXAN INDUCED DIABETIC RABBITS

Aspirin in the dose of 40 mg/kg potentiated the hypoglycaemic response of tolbutamide significantly after 5, 6 and 7 hours of drug administration in diabetic

TABLE. 12 : EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS (SINGLE DOSE) ON TOLBUTAMIDE HYPOGLYCAEMIA IN ALLOXAN INDUCED DIABETIC RABBITS.

Drugs (mg/kg in 2 % gum acacia)	Blood sugar change (in %) Mean ± S.E.					
	0 hour	3 hour	5 hour	7 hour	9 hour	11 hour
Tolbutamide (50)	100	69.2 ±2.05	72.41 ±3.73	95.94 ±2.28	101.48 ± 1.33	103.44 ± 2.11
Aspirin (40) + Tolbutamide (50)	100	93.89* ±1.86	61.69* ±5.46	87.66* ±1.75	100.45 ± 1.07	100.64 ± 1.42
Tannic (150) + Tolbutamide (50)	100	75.99 ±3.53	70.5 ±2.8	69.09 ±3.04	102.5 ± 0.57	101.21 ± 1.00
Tolmetin (20) + Tolbutamide (50)	100	69.63* ±1.89	61.33* ±2.7	87.35 ±4.49	100.15 ± 2.55	98.67 ±0.91

* indicates P value < 0.05.

EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS (SINGLE DOSE) ON TOLBUTAMIDE-HYPOGLYCAEMIA IN DIABETIC RABBITS.

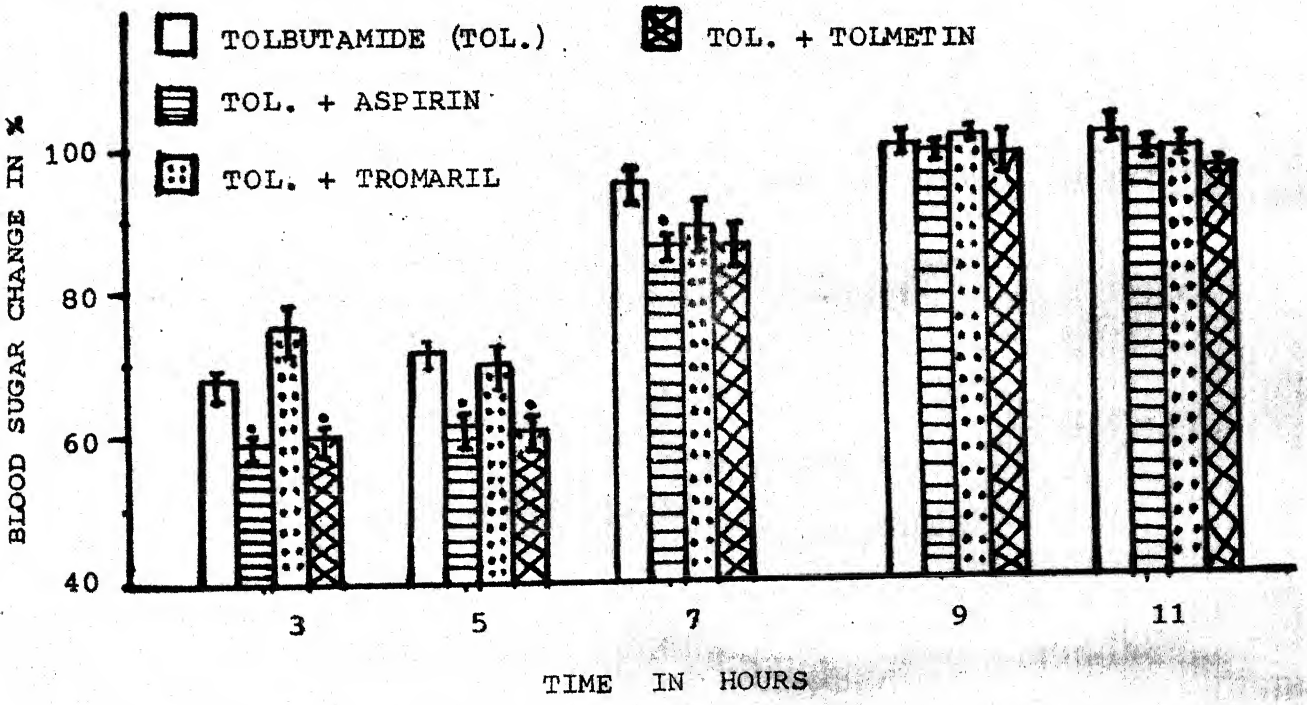


Fig. 11 : Shows effect of concurrent administration of anti-inflammatory agents on tolbutamide hypoglycaemia in diabetic rabbits. Aspirin and tolmetin show potention. • indicates P value < 0.05.

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-BLOCKERS (SINGLE DOSE) ON TOLBUTAMIDE-HYPOGLYCAEMIA IN DIABETIC RABBITS

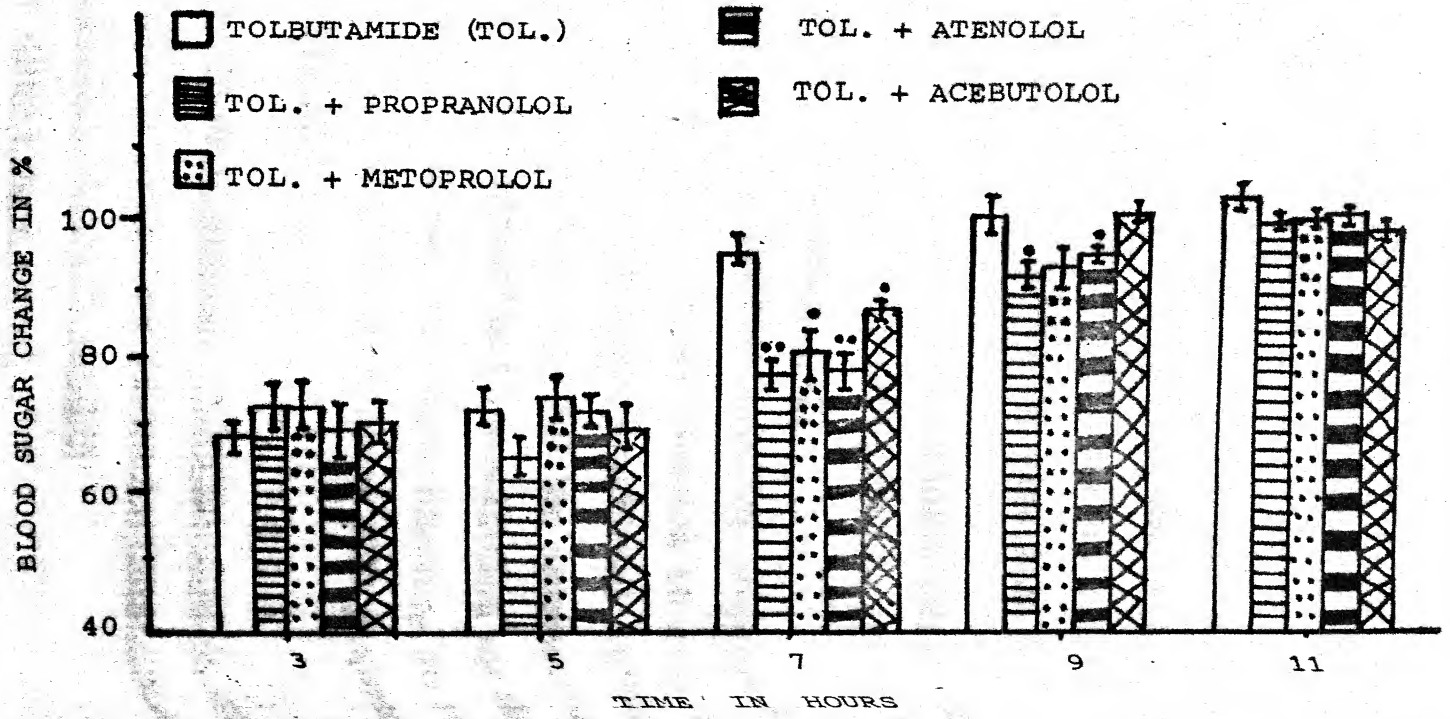


Fig. 12 : Shows effect of concurrent administration of beta-blockers on *tolbutamide hypoglycaemia* in diabetic rabbits, propranolol, atenolol and metoprolol show potention. ●, ●● indicate P value < 0.05 and < 0.01 respectively.

rabbits. Tolmetin (20 mg/kg) also enhanced the hypoglycaemic response of tolbutamide in diabetic rabbits. Enhancement of hypoglycaemia was significant only at 3 and 6 hours. Tramadol in the dose of 150 mg/kg did not significantly influence the tolbutamide induced hypoglycaemia (Table 12, Fig. 11).

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-ADRENERGIC BLOCKERS ON TOLBUTAMIDE-HYPOGLYCAEMIA IN ALLOXAN INDUCED DIABETIC RABBITS

In diabetic rabbits, propranolol (8 mg/kg), metoprolol (10 mg/kg), atenolol (6 mg/kg) as well as acebutolol (30 mg/kg), potentiated tolbutamide (80 mg/kg) induced hypoglycaemia. But the potentiation was delayed in nature. Significant potentiation was observed at 7 hours with all the drugs. However, the potentiation remained significant upto 9 hours with propranolol only (Table 13, Fig. 12).

EFFECTS OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON SERUM TOLBUTAMIDE CONCENTRATION AND BIOLOGICAL HALF-LIFE IN NORMAL RABBITS

(a) Single dose effect :

With concurrent administration of anti-inflammatory agents, the serum tolbutamide concentration after aspirin

TABLE 15 : EFFECT OF 7 DAYS PRETREATMENT OF ANTI-INFLAMMATORY AGENTS ON SERUM TOLBUTAMIDE CONCENTRATION AND HALF LIFE IN NORMAL RABBITS

Drugs (mg/kg in 2 % gum acacia	Serum tolbutamide concentration (in mg/dl) Mean ± S.E. 5 hour	7 hour	9 hour	11hour	Serum half life(hours)
2 % gum acacia(5ml) + Tolbutamide (50)	275.14 ± 6.01	225.11 ± 6.00	176.16 ± 8.53	137.09 ± 9.16	6.86 ± 1.21
Aspirin (40) + Tolbutamide (50)	260.38 ± 6.92	210.58 ± 7.3	180.11 ± 5.51	140.32 ± 6.89	6.47 ± 1.34
Ibuprofen (150) + Tolbutamide (50)	260.19 ± 4.39	219.33 ± 6.2	170.01 ± 6.78	130.60 ± 5.39	6.38 ± 1.18
Voltaretin (20) + Tolbutamide (50)	261.88 ± 5.85	200.8 ± 9.11	160.92 ± 6.97	130.8 ± 7.97	6.3 ± 1.43

EFFECT OF CONCURRENT ADMINISTRATION OF ANTI-INFLAMMATORY AGENTS ON SERUM TOLBUTAMIDE (T) CONCENTRATION.

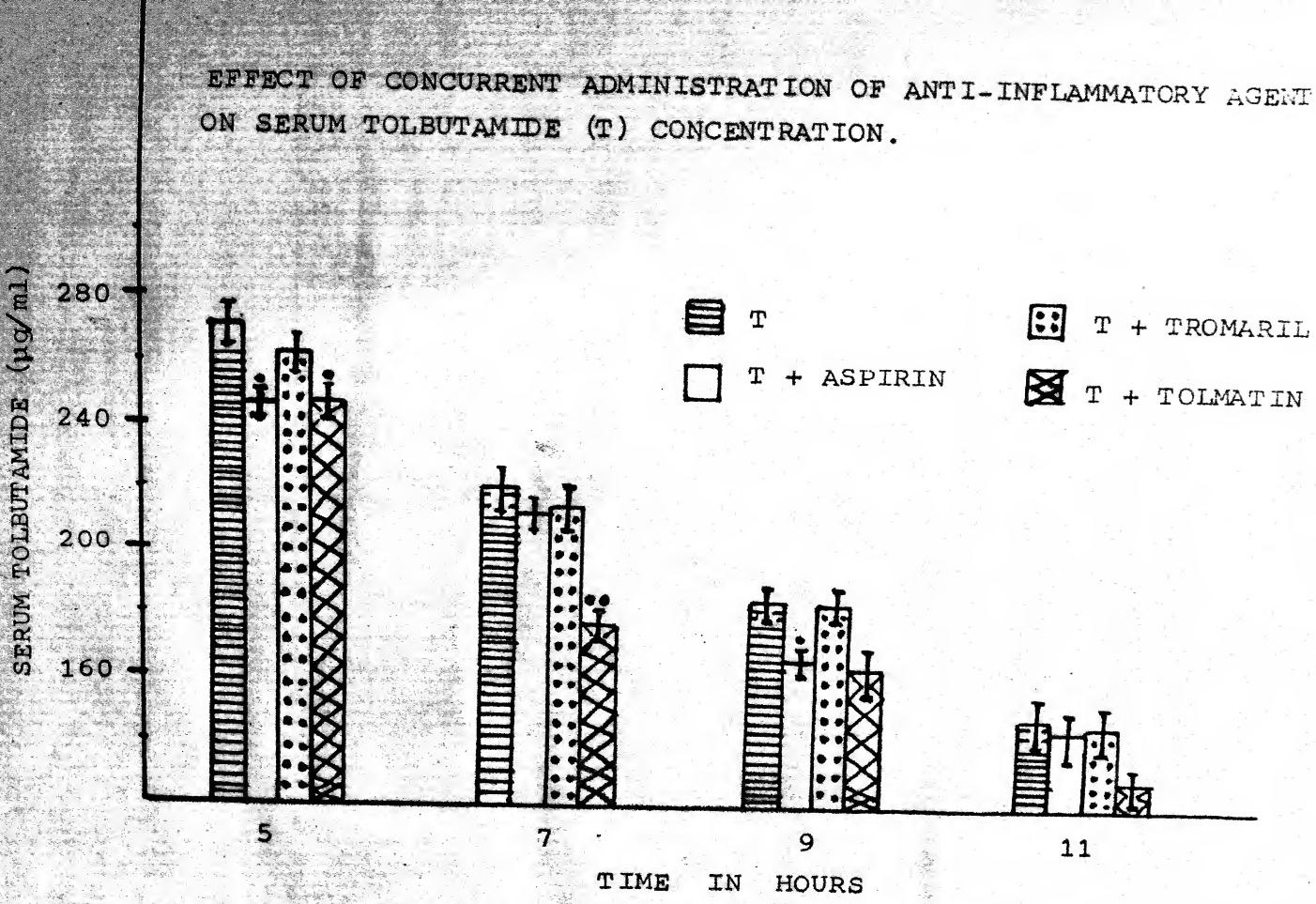


Fig. 13 : Shows effect of concurrent administration of anti-inflammatory agents on serum tolbutamide concentration in normal rabbits. Aspirin and tolmetin significantly reduced serum tolbutamide concentration. ● and ●● indicate P value < 0.05 and < 0.01 respectively.

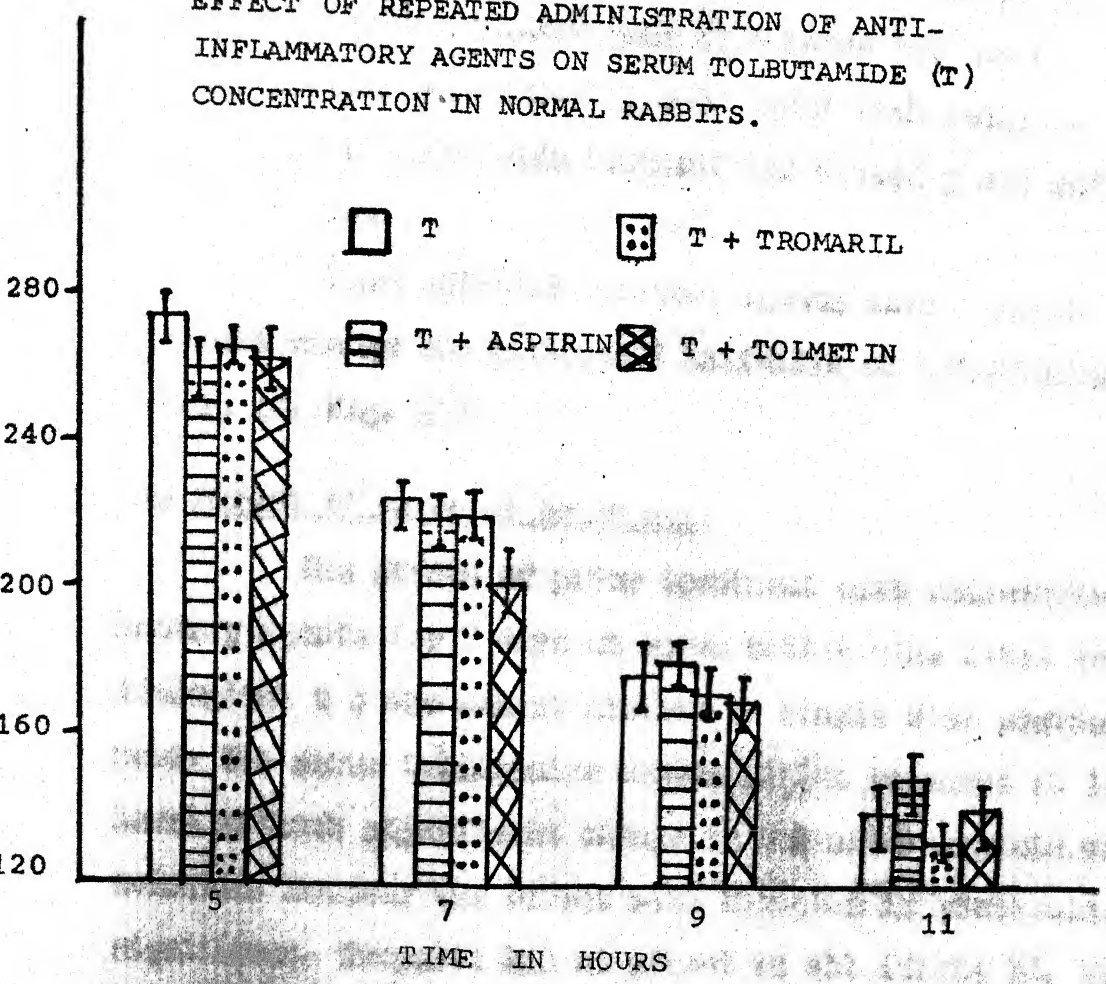


Fig. 14 : Shows effect of repeated administration (7 days) of anti-inflammatory agents on serum tolbutamide concentration in normal rabbits. Aspirin tolmetin or tromaril do not show any significant change in serum tolbutamide.

and tolmetin remained at low level compared to that of only tolbutamide and without any marked change after trumaril. In the control group tolbutamide reached a peak concentration (270.63 ± 5.64 ug/ml) at 5 hours. With anti-inflammatory drugs the peak time of serum tolbutamide level, although, remained same at 5 hours but serum concentrations were 246.67 ± 3.44 ug/ml with aspirin, 262.03 ± 6.12 ug/ml with trumaril and 247.41 ± 4.2 ug/ml with tolmetin.

These anti-inflammatory agents also didnnot markedly change the biological half-life of tolbutamide (Table 14, Fig. 13).

(b) Effect of repeated treatment:

The effect of prior treatment with anti-inflammatory agents for 7 days on serum tolbutamide level and biological t ½ are almost similar to single dose pretreatment. The serum tolbutamide concentration remained at low level without significant change in t ½ after aspirin and tolmetin. However the effect with tolmetin is statistically significant. Trumaril had no effect at all (Table 15, Fig.14)

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-ADRENERGIC BLOCKERS ON SERUM TOLBUTAMIDE CONCENTRATION AND BIOLOGICAL HALF-LIFE IN NORMAL RABBITS

(a) Single dose effects

The beta-blockers(Propranolol in

TABLE. 16 : EFFECT OF CONCURRENT DRUG ADMINISTRATION OF BETA BLOCKERS (SINGLE DOSE) ON SERUM TOLBUTAMIDE CONCENTRATION AND HALF LIFE IN NORMAL RABBITS.

Drugs (mg/kg in 2 % gum acacia	Serum tolbutamide concentration(ug/ml)Mean ± S.E.				Serum half life(hour)
	5 hour	7 hour	9 hour	11 hour	
Tolbutamide (50)	270.63 ± 8.64	220.61 ± 5.64	185.38 ± 6.30	147.5 ± 7.5	6.95 ± 1.2
Tolbutamide (50) + Propranolol (8)	260.38 ± 8.7	234.80 ± 8.52	201.61 ± 8.25	142.35 ± 6.09	6.7 ± 1.4
Tolbutamide (50) + Metoprolol (10)	264.02 ± 7.93	223.22 ± 9.85	178.88 ± 7.34	132.45 ± 5.81	6.3 ± 1.32
Tolbutamide (50) + Atenolol (6)	276.14 ± 11.06	246.41 ± 9.2	192.00 ± 4.34	159.21 ± 4.07	7.15 ± 1.00
Tolbutamide (50) + Acebutolol (30)	264.41 ± 8.25	221.20 ± 6.85	173.75 ± 9.26	132.2 ± 7.25	6.15 ± 1.6

TABLE. 17 : EFFECT OF 7 days PRETREATMENT OF BETA-ADRENERGIC BLOCKERS ON TOLBUTAMIDE SERUM CONCENTRATION AND HALF LIVE IN NORMAL RABBITS.

Drugs(mg/kg in 2 % gum acacia	Serum tolbutamide concentration(in mcg%) Mean ± S.E.				Serum half life(hour)
	5 hour	7 hour	9 hour	11 hour	
2% gum acacia + Tolbutamide (50)	275.13 ± 6.01	225.14 ± 7.91	176.16 ± 8.53	137.09 ± 4.16	6.26 ± 1.21
Propranolol (8) + Tolbutamide (50)	265.00 ± 5.56	222.35 ± 6.7	180.76 ± 4.51	152.30 ± 9.85	6.40 ± 1.85
Metoprolol (10) + Tolbutamide (50)	265.92 ± 9.59	210.71 ± 5.56	171.39 ± 9.63	138.31 ± 4.36	6.01 ± 0.92
Atenolol (6) + Tolbutamide (50)	271.25 ± 8.5	209.05 ± 5.6	160.0 ± 7.32	130.54 ± 7.8	5.9 ± 0.78
Acebutolol (30) + Tolbutamide (50)	266.95 ± 9.45	210.54 ± 9.51	170.11 ± 7.38	138.09 ± 9.12	6.00 ± 1.46

TABLE. 19 : EFFECT OF CONCURRENT IVGG ADMINISTRATION OF ANTI-TUBERCULOUS AGENTS(SINGLE DOSE) ON SERUM TOLBUTAMIDE CONCENTRATION AND HALF LIFE IN ALLOXAN INDUCED DIABETIC RABBITS.

Drugs (mg/kg in 2 % gum acacia)	Serum tolbutamide concentration (in mg/dl) Mean ± S.E.				Serum half life(hour)
	5 hour	7 hour	9 hour	11 hour	
Tolbutamide (50)	272.02 ± 5.06	213.19 ± 8.03	180.99 ± 6.25	150.92 ± 5.07	6.00 ± 1.30
Tolbutamide (50) + Aspirin (40)	264.40 ± 8.99	202.28 ± 6.91	166.1 ± 8.07	145.96 ± 7.13	6.7 ± 1.4
Tolbutamide (50) + Tremexil (150)	277.61 ± 6.02	225.98 ± 7.66	189.16 ± 9.13	135.38 ± 6.78	6.00 ± 1.21
Tolbutamide (50) + Tolmetin (20)	260.14 ± 9.37	205.85 ± 5.83	161.92 ± 9.49	133.21 ± 6.58	6.2 ± 1.08

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-BLOCKERS ON SERUM TOLBUTAMIDE LEVEL IN NORMAL RABBITS.

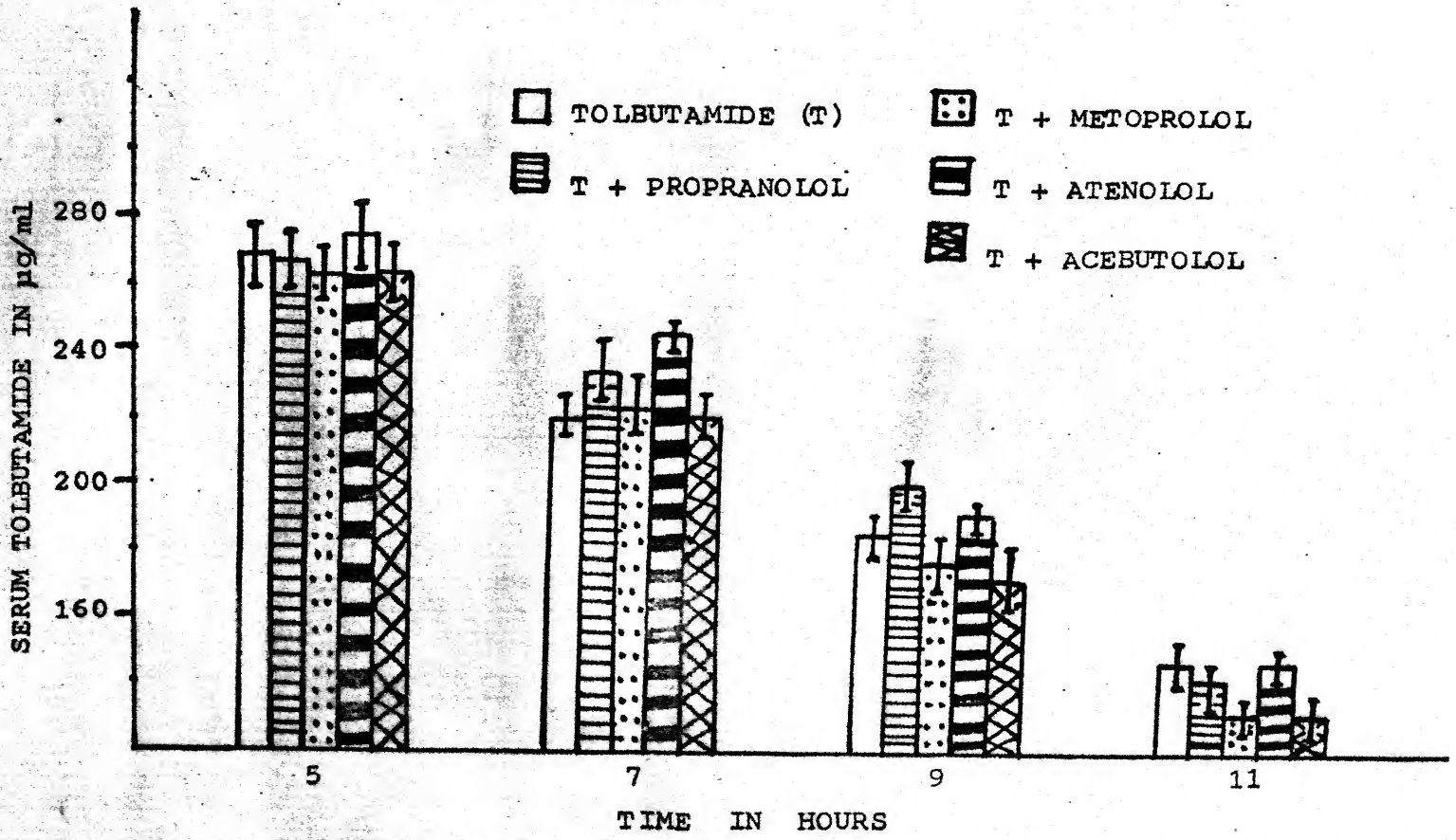


Fig. 15 : Shows effect of concurrent administration of beta-blockers on serum tolbutamide level in normal rabbits. Propranolol, metoprolol, atenolol and acebutolol do not show any significant change.

EFFECT OF REPEATED ADMINISTRATION OF BETA-BLOCKERS ON SERUM TOLBUTAMIDE CONCENTRATION IN NORMAL RABBITS.

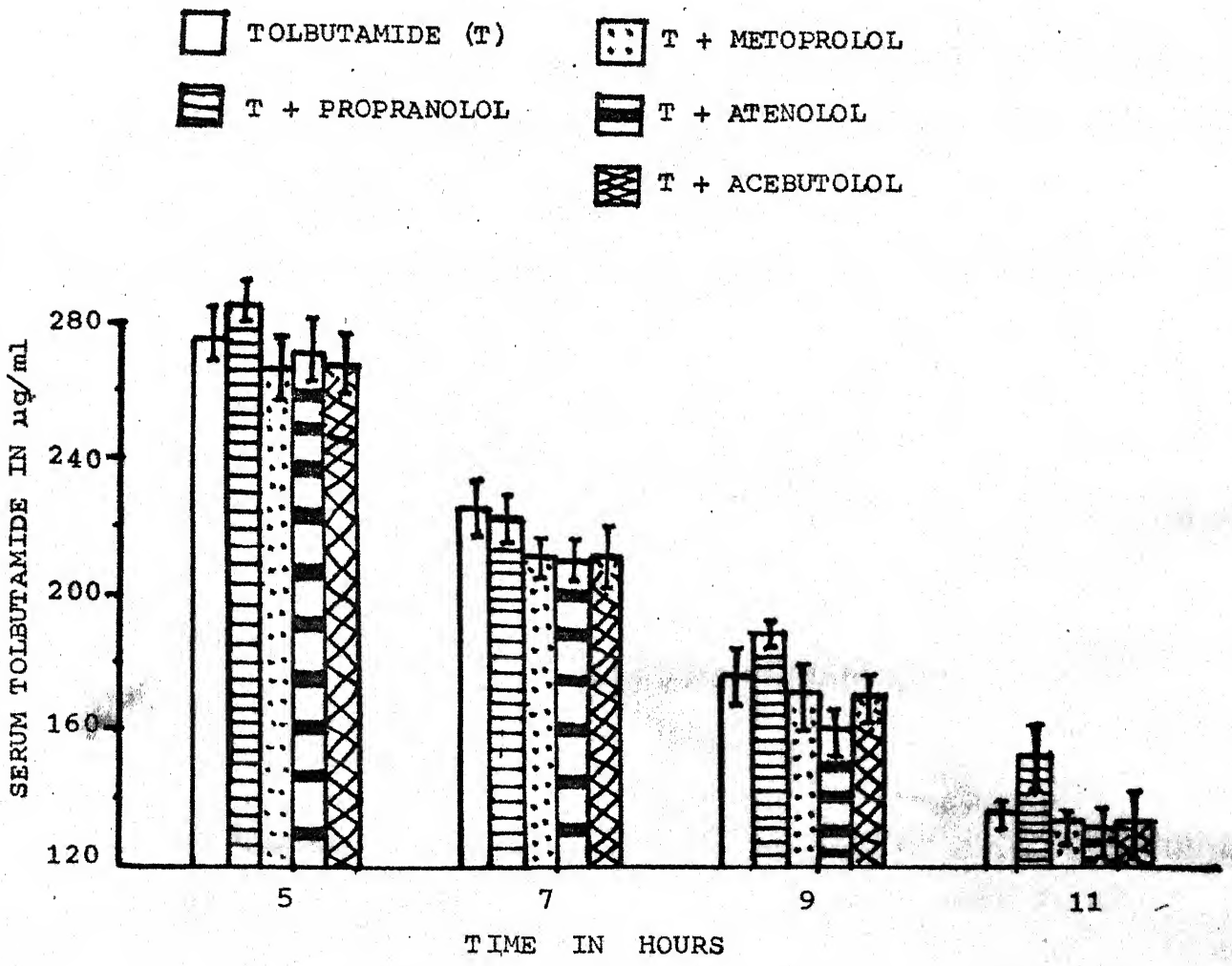


Fig. 16 : Shows effect of repeated administration of beta-blockers (7 days) on serum tolbutamide concentrations. Propronolol, metoprolol, atenolol or acebutolol do not show any effect.

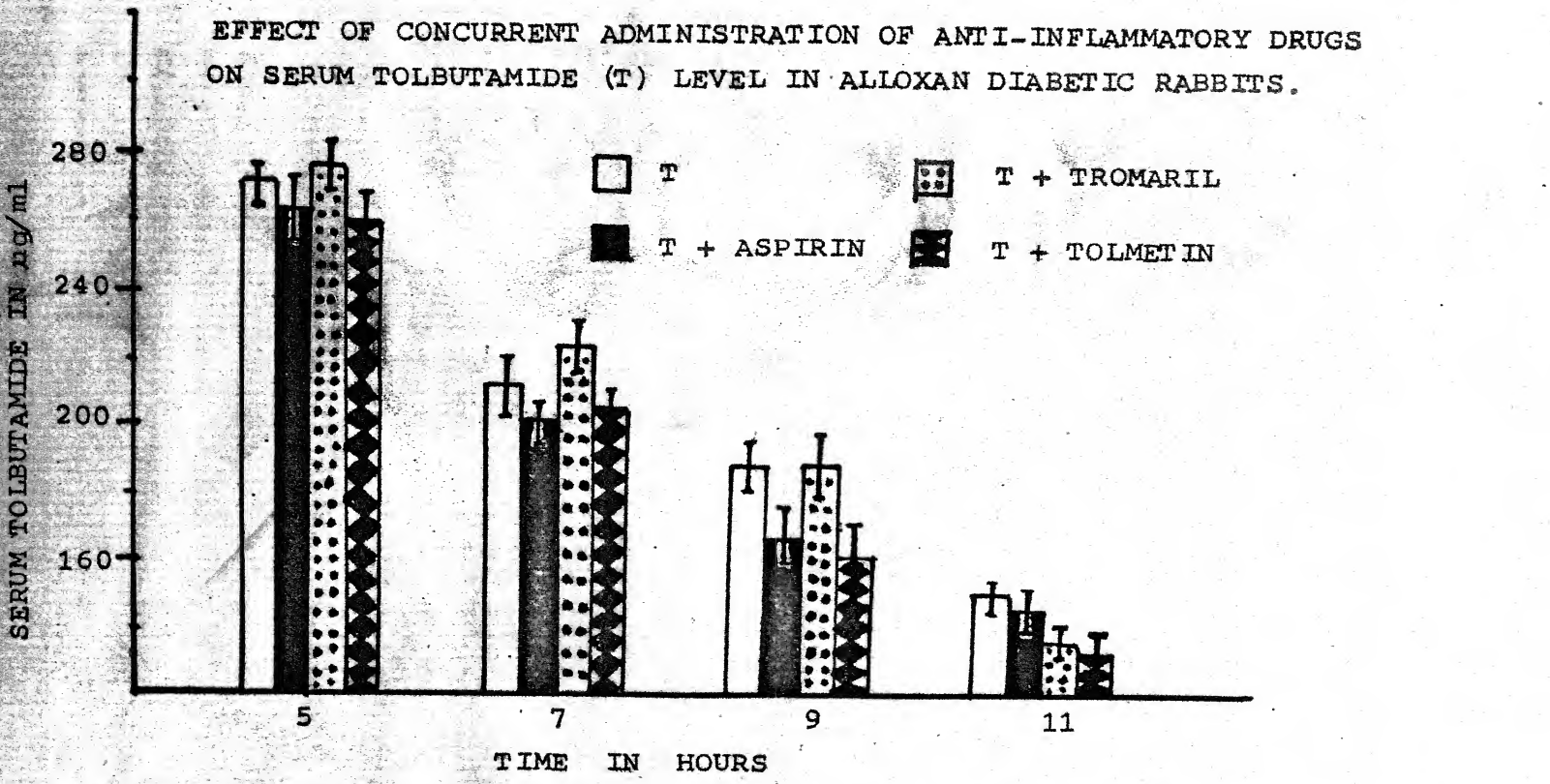


Fig. 17 : Shows the effect of concurrent administration of anti-inflammatory drugs on serum tolbutamide level in diabetic rabbits. Aspirin, tromaril or tolmetin do not show any significant change in serum tolbutamide concentration.

EFFECT OF CONCURRENT ADMINISTRATION OF BETA-BLOCKERS ON SERUM TOLBUTAMIDE LEVEL IN ALLOXAN DIABETIC RABBITS.

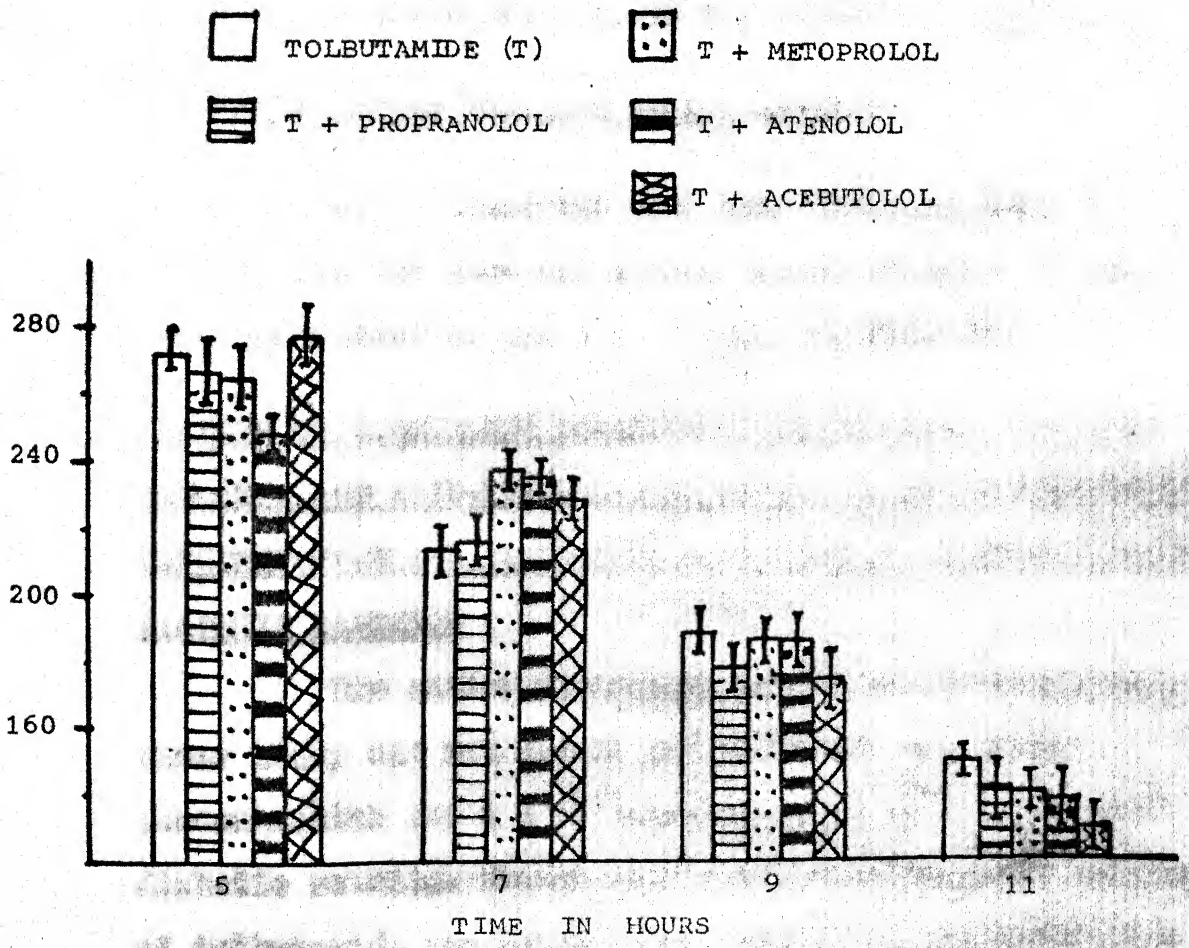


Fig. 18 : Shows the effect of concurrent administration of beta-blockers on serum tolbutamide level in alloxan-induced diabetic rabbits. No significant change occurred after the administration of propranolol, metoprolol, atenolol & acebutolol.

DISCUSSION

D I S C U S S I O N

In the treatment of diabetes mellitus the non-hormonal hypoglycaemic agents are of the great importance because of convenience of administration and low cost of treatment since these agents are orally effective. Sulphonylureas still continue to be the mainstay in the treatment of maturity onset diabetes. Since, the discovery of sulphonylureas as a potential group of orally effective hypoglycaemic agents a large number of derivatives have been synthesized, tested and clinically introduced in therapy, tolbutamide is the oldest sulphonylurea and it still finds favour from physician due to high margin of safety and low incidence of side effects. The newer sulphonylureas, although, similar to tolbutamide in mechanism of action and clinical efficacy but enjoy additional superiority primarily due to longer duration of action and thus less frequency of administration (chlorpropamide once a day, glibenclamide once or twice a day and tolbutamide 3-4 times a day). But many clinicians still believe administering a hypoglycaemic agent with each meal of day and consider to be more effective to maintain normal blood sugar level than longer acting drugs.

High incidence of cardiovascular diseases in general and hypertension and coronary diseases in particular in diabetes mellitus is well documented(Clawson and Bell,

1949). Beta-adrenergic blocking agents are a major group of drugs in the management of cardiovascular diseases in the present clinical practice. Thus use of beta-blockers in diabetic patients with cardiovascular complications is quite common. These drugs also possess certain effect on glucose metabolism and effect blood sugar level(Kotler et al., 1966). It is therfore very likely that beta-blocker is concurrently administered to a diabetic patient and it may affect the response of an antidiabetic agent used. Although a large number of evidence of adverse drug interactions with tolbutamide and various beta-blocker have accummulated but still it is difficult to draw a definite conclusion about mode of concurrent therapy with these groups of drugs. It is so because beta-blockers with selective action are being introduced and it is after some time that their interaction potential with other drgus is brought to light. It was, therefore felt worthwhile to conduct further drug interaction studies in animals between beta-blockers and tolbutamide.

Anti-inflammatory analgesics are also a very common group of drugs in the symptomatic relief of musculoskeletal pain and arevery frequently prescribed in all patients. Use of anti-inflammatory agents is also associated with disturbances in blood sugar level and thus they also

CORRELATION BETWEEN HYPOGLYCAEMIC RESPONSE AND SERUM CONCENTRATION OF TOLBUTAMIDE.

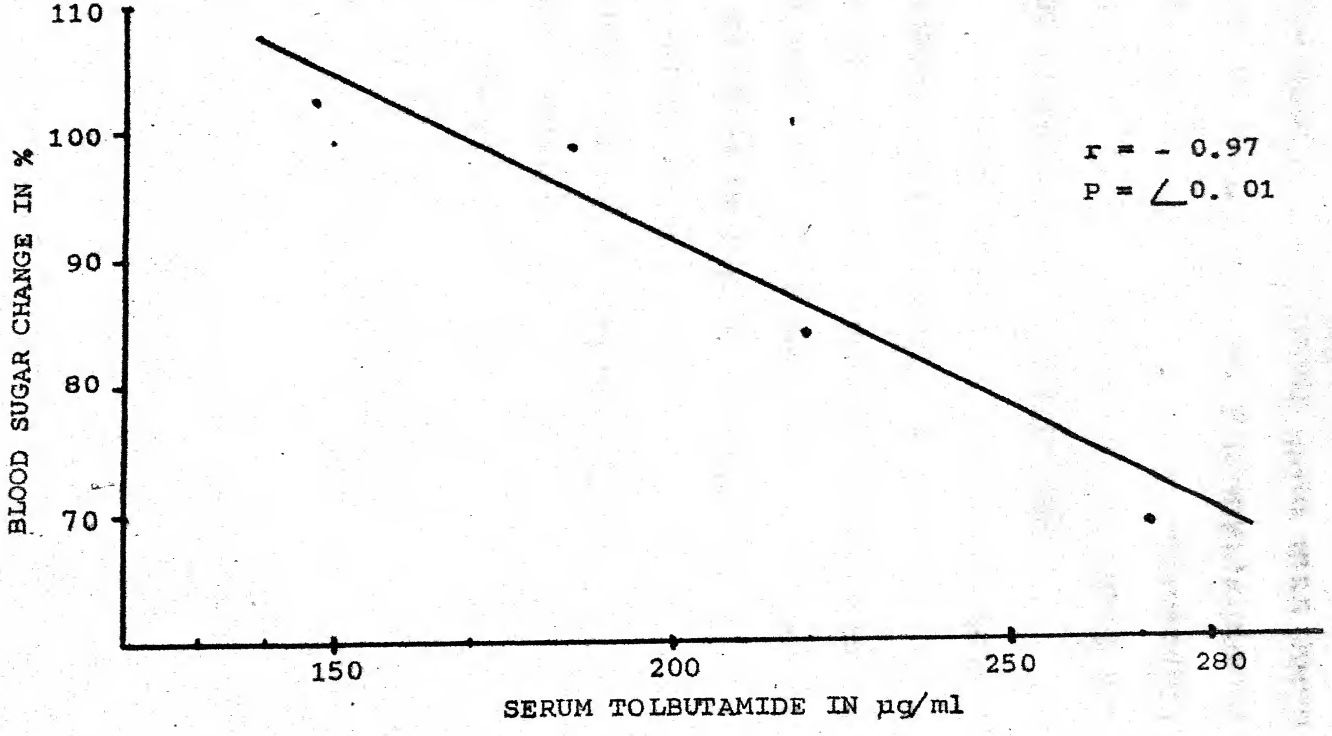


Fig. 19 : Shows regression line between blood sugar change and serum concentration of tolbutamide (50 mg/kg) in normal rabbits. The correlation coefficient (r = -0.97) is statistically significant (P ∠ 0.001)

influence blood sugar control by oral anti-diabetics.

In the present study beta-blockers and anti-inflammatory drugs were included in the interaction study with tolbutamide, amongst the beta-blockers the cardio-selective and among the anti-inflammatory drugs the newly introduced nonsteroidal agents were chosen as drug interaction studies with them are quite limited.

In this investigation tolbutamide was found to produce a dose dependent hypoglycaemic effect in normal as well as in alloxan induced diabetic rabbits. However, the effects were qualitatively and quantitatively similar in both types of animals excepting an earlier peak response in diabetic animals(3hours) as compared to normal animals (6 hours). Tolbutamide at an oral dose of 60mg/kg produced a marked hypoglycaemia (about 60 %) in the normal and diabetic animals at maximal hypoglycaemic response and this effect persisted over nine hours.

The extent of hypoglycaemia produced by tolbutamide was found to be dependent on the blood concentration of tolbutamide attained. At the peak response the tolbutamide concentration was the highest and it gradually diminished along with serum tolbutamide concentration (Fig. 19). In addition further information could be deduced from the

present study about the minimum serum level of tolbutamide required to induce and maintain the pharmacologic response. Our data showed that after 9 hours of administration of tolbutamide the blood sugar level returned(98.8 %, in normal rabbits and 101 %, in diabetic rabbits) to normal with serum level of 185.38 ± 6.3 in normal rabbits and 188.99 ± 6.25 mg/ml in diabetic rabbits. The serum concentration of tolbutamide less than 185 - 190 mg/ml seemed to be ineffective to evoke hypoglycaemic response.

Aspirin, tolmetin and tromaril have been used at doses less than their $E.D._{50}$ doses. The anti-inflammatory $E.D._{50}$ values for aspirin, tolmetin and tromaril are, 98, 40 and 132 mg/kg respectively(Shadma, 1983). Aspirin (40 mg/kg) and tolmetin (20 mg/kg) per se produced significant hypoglycaemia whereas tromaril (100 mg/kg) did not show this effect on blood sugar level. The anti-inflammatory agents produce anti-inflammatory action through a common mechanism of prostoglandin synthetase inhibition (Ferreira et al, 1971; Vane, 1971) but the descrepancy on blood sugar changes by these agents is difficult to explain. However, aspirin produces hypoglycaemia(Menston, 1979) or hyperglycaemia (Flower et al, 1980) in toxic doses. In this study aspirin in therapeutic doses produced hypoglycaemia. Phenylbutazone and indomethacin inspite of being potent anti-infl-

amatory drugs do not change blood sugar level to any significant extent (Rothermich, 1966; Sharma et al., 1981) Prostaglandins are known to exert insulin like action (Nakano, 1973). Anti-inflammatory agents by prostaglandin synthesis inhibition are rather theoritically excepted to raise blood sugar level by anti-insulin effect. It seems that hypoglycaemia induced by some anti-inflammatory agents is probably not related to prostaglandin synthesis inhibition. The underlying mechanism for the effect is unclear and requires further investigations for elucidation.

Propranolol is a nonselective beta-blocker but metoprolol, atenolol and acebutolol are cardioselective (β_1) beta-receptor blocking agents (Weiner, 1980). Recent studies reveal that β_2 receptors present in liver and pancreatic beta-cells of Langerhans are involved in catecholamine mediated effects on glucose metabolism (Weiner, 1980) and insulin release. Nonselective beta-adrenergic blocking agents considerably modify glucose metabolism by inhibiting metabolic β_2 receptors, whereas cardioselective drugs are said to be free from metabolic effect. In this study propranolol produced hypoglycaemia. This observation is in agreement with earlier reports(McAury, 1974). Metoprolol and acebutolol did not show any marked effect on blood sugar level. This finding again confirms the

noninvolvement of cardioselective beta-blockers in glucose metabolism (Heasman, 1976). However, atenolol another cardioselective beta-blocker exhibited hypoglycaemic response.

Aspirin and tolmetin when administered along with tolbutamide increased tolbutamide hypoglycaemia in normal as well as alloxan induced diabetic rabbits but interestingly the serum tolbutamide concentration was found significantly lower than the corresponding normal values. This clearly indicates that the potentiation of hypoglycaemia by the anti-inflammatory drugs under study is not by enhancing tolbutamide bioavailability.

On the contrary, these anti-inflammatory agents decreased serum tolbutamide level by some mechanism most probably by decreasing absorption of tolbutamide. However, earlier reports mention that salicylates displace tolbutamide from plasma-protien binding sites and thus increase unbound sulphonylureas in the blood(Hansten, 1979). Moreover Lower et al. (1980) have suggested that the potentiation is due to intrinsic hypoglycaemic action of anti-inflammatory drugs. Our findings also confirm this contention as aspirin and tolmetin per se produced hypoglycaemia. Furthermore, it is reasonable to presume that if the anti-inflammatory agents had not decreased the tolbutamide bioavailability the hypoglycaemic potentiation would have been still more. Therefore,

it is probable that the hypoglycaemic potentiation might be partly due to intrinsic hypoglycaemic action of anti-inflammatory drugs. The lowering of serum concentration of tolbutamide appears to be due to decreased absorption by anti-inflammatory drugs but it requires further confirmation.

The other anti-inflammatory agent tremaril was found not to have any intrinsic hypoglycaemic action or any effect on serum tolbutamide concentration. Tremaril did not produce any effect on tolbutamide hypoglycaemia.

Pretreatment with aspirin and tolmetin daily for a week but without concurrent administration with tolbutamide on the 8th day were found to increase tolbutamide hypoglycaemia without any significant change in serum tolbutamide concentration. Moreover, in the control group, the hypoglycaemic effect of aspirin and tolmetin remained persistent on the 8th day. Since these drugs did not change tolbutamide concentration significantly their effect on absorption, metabolism and excretion of tolbutamide is out of question. The possible mechanism of this potentiation might be due to persistent hypoglycaemic action of aspirin and tolmetin after prolonged treatment.

Propranolol and atenolol, among the beta-blocking drugs potentiated tolbutamide hypoglycaemia in normal as well as in diabetic rabbits. These drugs had no effect on

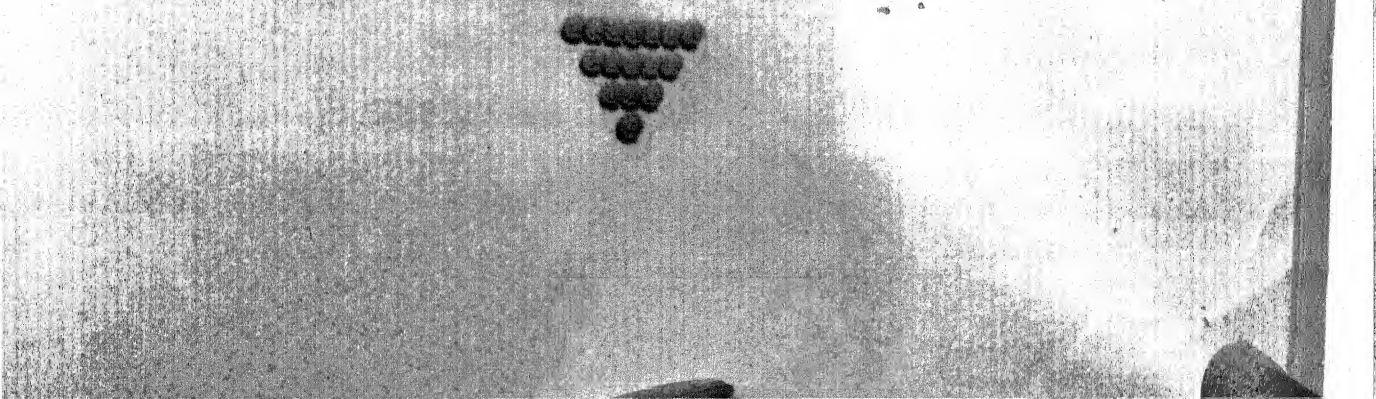
serum tolbutamide concentration pattern. It appears that the tolbutamide hypoglycaemia potentiation by beta-blockers might be due to their hypoglycaemic action through metabolic B_2 receptor blockade. But atenolol has been reported to be a selective cardiac B_1 blocker. Thus it is not expected to produce hypoglycaemia or potentiate sulphonylurea induced hypoglycaemia. In our study atenolol produced these effects. It does possess intrinsic hypoglycaemic action which may not be B-receptor mediated.

Metoprolol and acebutolol, the other two cardio-selective beta-blockers, although did not produce any effect in normal rabbits but potentiated tolbutamide hypoglycaemia in diabetic rabbits. It can be concluded that intrinsic hypoglycaemia in diabetic action and potentiation of tolbutamide hypoglycaemia by beta-receptor blockers is due to metabolic B_2 - receptor blockade. Although atenolol, metoprolol and acebutolol are selective B_1-blockers but a minor B_2 blocking activity in these drugs can not be completely ruled out. This study further shows that metoprolol and acebutolol are more selective than atenolol.

Propranolol after a week-long treatment potentiated the tolbutamide hypoglycaemia on the 8th day. But in the control group the blood sugar level remained with in normal range. Thus it appears the mechanism of potentiation

is not due to persistent hypoglycaemic action of propranolol. Moreover, serum tolbutamide level was also not markedly changed. Therefore propranolol after prolonged treatment might not be affecting tolbutamide absorption metabolism or excretion. From the present data it is not possible to explain the exact mechanism how chronic treatment with propranolol potentiated tolbutamide hypoglycaemia. There are many possibilities including increased insulin release by tolbutamide due to some cellular change produced by chronic pretreatment with propranolol.

It can be concluded from the present investigation that anti-inflammatory and beta-blocking drugs when administered along with tolbutamide may give rise to therapeutic problems. Concurrent administration of these drugs with tolbutamide can lead to improper control of diabetes and in higher doses may lead to dangerous hypoglycaemia. But tromexil and cardioselective beta-blockers like metoprolol and acebutolol are comparatively safer in this respect.



CONCLUSION

C O N C L U S I O N

In the present study effects of concurrent and prior repeated treatment for a week with certain anti-inflammatory and beta-adrenoceptor blocking agents on tolbutamide-induced hypoglycaemia in normal healthy as well as alloxan-induced diabetic rabbits were investigated. Among the anti-inflammatory drugs aspirin the lodest and tolmetin and tromaril the comparatively newly introduced nonsteroidal anti-inflammatory agents and among the beta-adrenoceptor blockers propranolol the non-selective and atenolol, metoprolol and acebutolol the selective cardiac (B_1) receptor blockers were chosen for the interaction study with tolbutamide. In order to determine the mechanism of interaction serum tolbutamide concentration was also measured along with blood sugar estimation.

From the results obtained the following conclusions can be drawn.

1. Our experiments show that tolbutamide produces a dose-dependent hypoglycaemic action with a peak response at 6 hours in normal rabbits and 3 hours in diabetic rabbits and the effect remains persistent over 9 hours. The corresponding serum

tolbutamide concentration has a significant correlation with blood sugar changes (fig. 19)

2. Aspirin, tolmetin, propranolol and atenolol seem to have intrinsic hypoglycaemic effect whereas tromaril, metoprolol and acebutolol did did not produce any significant change on blood sugar level.

3. Out of three anti-inflammatory agents under study aspirin and tolmetin on concurrent administration and prior 7 days treatment were found to potentiate the tolbutamide-induced hypoglycaemia in normal as well as diabetic rabbits with corresponding decrease in serum tolbutamide level. However, tromaril neither potentiated hypoglycaemia nor changed serum tolbutamide level pattern.

4. Since aspirin and tolmetin decreased serum tolbutamide levels the potentiation of tolbutamide-hypoglycaemia is probably due to their intrinsic hypoglycaemic action and not due to pharmacokinetic alterations.

5. Propranolol and atenolol when administered along with tolbutamide increased tolbutamide hypoglycaemic response without any effect on serum tolbutamide concentration and tolbutamide half-life

in normal rabbits. But metaprolol and acebutolol did not influence tolbutamide hypoglycaemia and its serum level to any extent. But in diabetic rabbits all beta-blockers somehow potentiated tolbutamide hypoglycaemia.

6. In normal rabbits pretreated with beta-blockers for 7 days only propranolol and atenolol potentiated tolbutamide hypoglycaemia. However atenolol showed a delayed response but metoprolol and acebutolol had no effect. The serum tolbutamide concentration remained unchanged.

7. It can be concluded that use of aspirin, tolmetin, propranolol, atenolol, metoprolol and acebutolol in diabetic individuals kept on tolbutamide treatment can increase changes of tolbutamide hypoglycaemic episodes. Therefore due precautions should be taken to prevent such episodes by suitable dose adjustments or selecting alternative drugs for simultaneous treatment of cardiovascular or inflammatory conditions. However, tromaril is preferable than other anti-inflammatory agents for simultaneous use with tolbutamide. All the beta-blockers are potentially dangerous although cardioselective drugs preferably metoprolol and acebutolol can be used carefully if use of a beta-blocker is needed.

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